

Appl. No. To Be Assigned; Group Art Unit: To Be Assigned
 Dkt. No. 0610.0050001/MAC;
 Inventor(s): Robinson et al.; Tel: 202/371-2600
 Title: Modular Assembly of Antibody Genes, Antibodies
 Prepared Thereby and Use

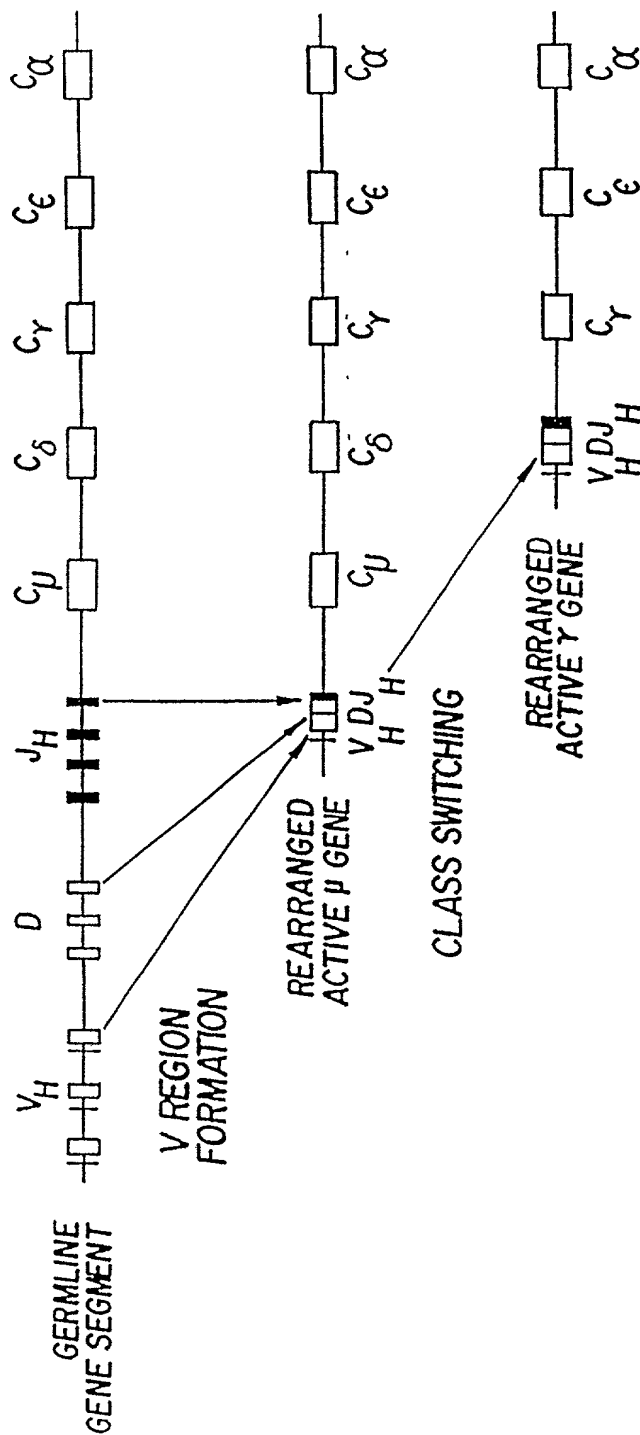


FIG. 1

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Ig heavy chain J-C regionhuman heavy chain J regions

J|CH1

JH1 GCTGAATACTTCCAGCACTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCAG
 JH2 CTACTGGTACTTCGATCTCTGGGGCCGTGGCACCCTGGTCACTGTCTCCTCAG
 JH3 ATGCTTTTGATGTCTGGGGCCAAGGGACAATGGTCACCGTCTCCTCAG
 JH4 ACTACTTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAG
 JH5 ACACTGGTTCGACTCCTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAG
 JH6 AT(TAC)₅GGTATGGACGTCTGGGGGCAAGGGACCACGGTCACCGTCTCCTCAG
Consensus TCGACCTCTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCAG

mouse heavy chain J regions

J|CH1

JH1 TACTGGTACTTCGATGTCTGGGGCCAGGGACCACGGTCACCGTCTCCTCAG
 JH2 TACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAG
 JH3 CCTGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAG
 JH4 TACTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAG
Consensus TTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCAG

Ig light chain J-C regionhuman Kappa J region

J|c

JK1 GGACGTTCCGCCAAGGGACCAAGGTGGAAATCAAAC
 JK2 ACACTTTTCGCCAGGGGACCAAGCTGGAGATCAAAC
 JK3 TCACTTTCGGCCCTGGGACCAAGTGGATATCAAAC
 JK4 TCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAAC
 JK5 TCACCTTCGGCCAAGGGACACGACTGGAGATTAAC
Consensus TTCGCCAAGGGACCAAGGTGGAGATCAAAC

mouse Kappa J region

J|c

JK1 TGGACGTTCCGTTGGAGGCACCAAGCTGGAAATCAAAC
 JK2 TACACGTTCCGAGGGGGGACCAAGCTGGAAATAAAAC
 JK3 TTCACATTCACTGATGGGACCAGACTGGAAATAAAAC
 JK4 TTCACGTTCCGCTCGGGGACAACTGGAAATAAAAC
 JK5 CTCACGTTCCGTTGCTGGGACCAAGCTGGAGCTGAAAC
Consensus TTCGTTGGGGGACCAAGCTGGAAATAAAAC
 UIG[MJK] TGGTTCGACCTTTATTTTG

human Lambda pseudo J region

J|c

JPSL1 CACATGTTTGGCAGCAAGACCCAGCCCACTGTCTTAG

mouse Lambda J region

J|c

JL1 TGGGTGTTCCGTTGGAGGAACCAAACTGACTGTCCTAG
 JL2 TATGTTTTTCGGCGGTGGAACCAAGGTCACTGTCCTAG
 JL3 TTTATTTTTCGGCAGTGAACCAAGGTCACTGTCCTAG
Consensus TTCGGCGGTGGAACCAAGGTCACTGTCCTAG

FIG. 2

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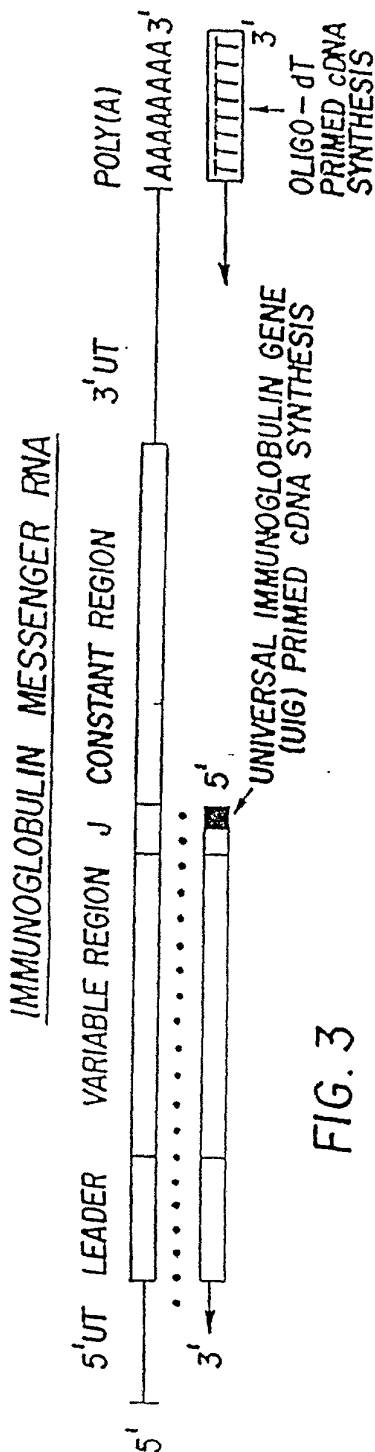


FIG. 3

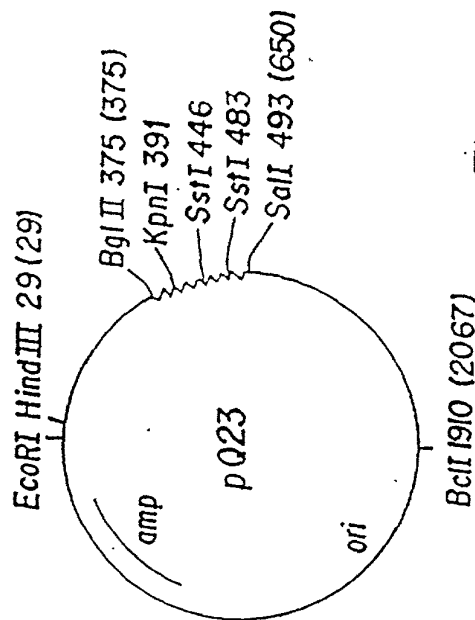


FIG. 5

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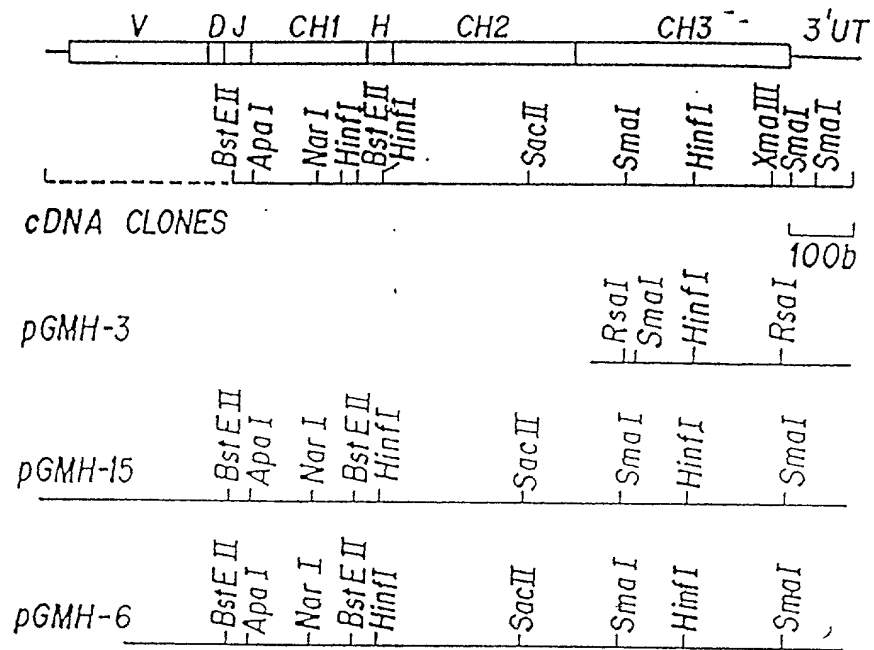


FIG. 4A

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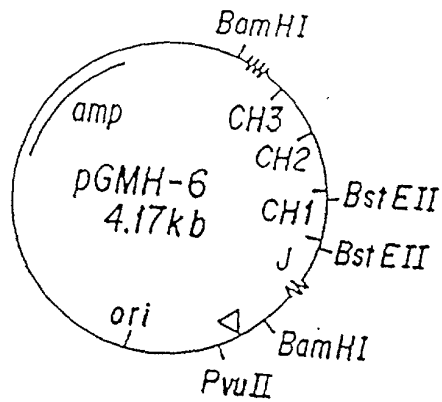


FIG. 4B

206070 "54624007

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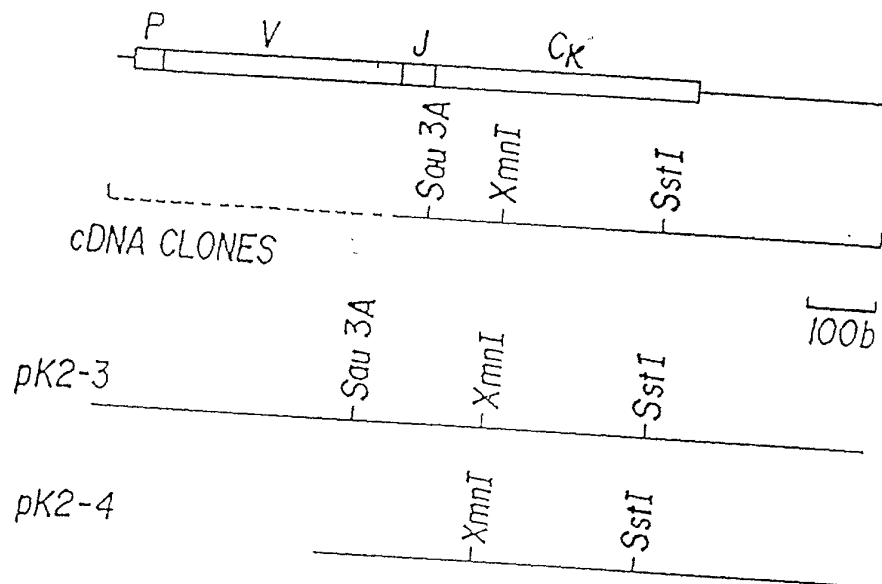


FIG. 6A

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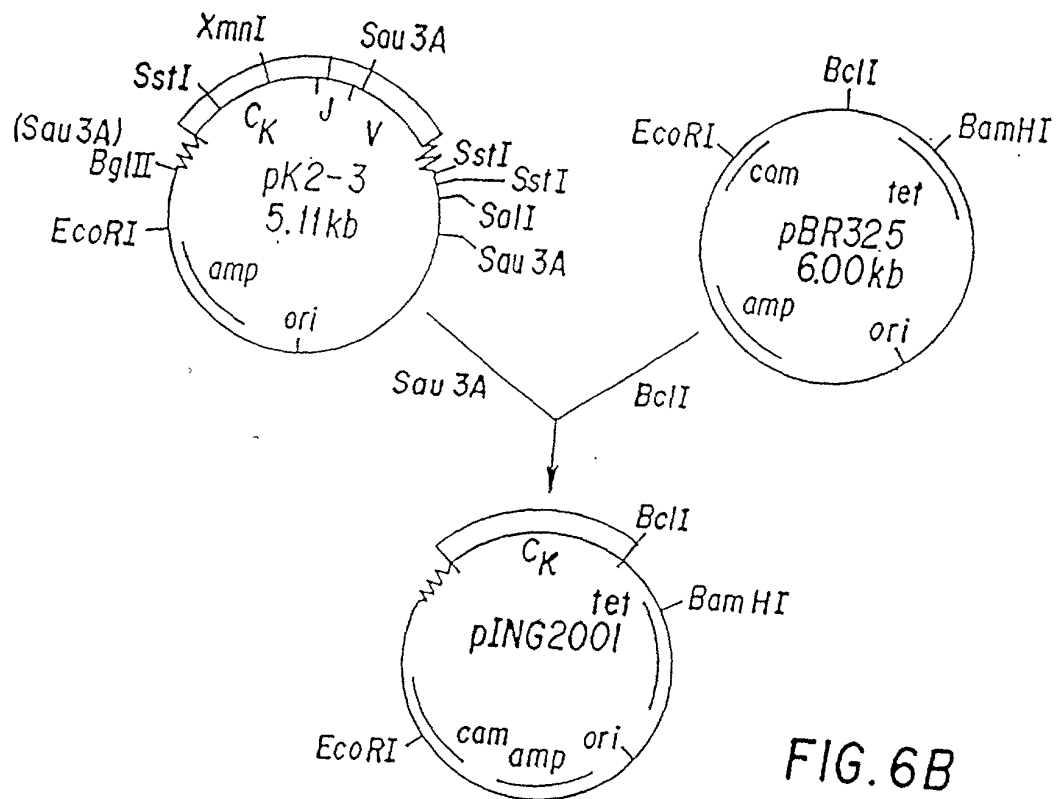


FIG. 6B

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Ig HEAVY CHAIN J-C REGION

— J REGION —|— IgG1 CH1 REGION —
HUMAN IgG1 pGMH-6
GGTCACCGTCTCCTCAG CCTCCACCAAGGCCCATC
BstEII

MOUSE HEAVY CHAIN J REGIONS AND PRIMERS

	MISMATCHES				
	N	JH1	JH2	JH3	JH4
JH1 TACTGGTACTTCGATGCTCTGGGGCCAGGACCAACGGTCACCGTCTCCTCAG [MJH1] GCCAGTGGCAGAGGAGTCGGT	21	0	4	4	1
JH2 TACTTTGACTACTGGGGCCAGGACCACTCTCAGAGTCTCCTCAG [MJ21] GAGAGTGTACAGCAGTCCGT	21	4	1	7	4
JH3 CCTGGTTTGCTTACTGGGGCCAGGACTCTGGTCACTGTCTCTGCAG [MJH3] ACCAGTCACAGACGTCGGT TCCCTGAGACCACTGGCAGAG [MJH3-BSTEII] [MJH3-BSTEII(13)] ACCAGTGGCAGAG BstEII	21 21 13	4 3 1	7 7 4	0 1 1	5 5 2
JH4 TACTATGCTATGGACTACTGGGGTCAAGAACCTCAGTCACCGTCTCCTCAG [MJH4] GTCAGTGGCAGAGGAGTCGGT	21	1	4	5	0

FIG.7A

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Ig KAPPA CHAIN J-C REGION

-J REGION ———-IgK CONSTANT REGION———
CTGGAGATGAAAC GAACTGTGGCTGCACCATCTGCTTCACTTCCC
TGATCAAAC GAACTGTGGCTGCACCATCTGCTTCACTTCCC
BclI

MOUSE HEAVY KAPPA J-REGIONS AND PRIMERS

JK1 TGGACGTTCCGTGGAGGCACCAAGCTGGAATCAAAC
[5JK1] GCAAGCCACCTCCGTGG

JK2 TACACGTTCCGAGGGGGACCAAGCTGGAATCAAAC
[JK2BGLII] CCCGTGGCTGACCTCTAGATT
[5JK2] GTGCAAGCCTCCCCCTGG BgIII

JK4 TTCACGTTCCGCTCGGGGACAAAGTTGGAATCAAAC
[5JK4] GCAAGCCGAGCCCTGT
[JK4BGLII] GCGGCTGTTCAACCTCTAGATT
BgIII

JK5 CTCACGTTCCGTGGTGGACCAAGCTGAGCTGAAAC
[5JK5] GCAAGCCACGACCTGG

[MJK] TGGTTCGACCTTTATTTTG

MISMATCHES

N	JK1	JK2	JK4	JK5
17	0	3	6	3
21	3	3	5	3
17	6	4	0	4
23	7	6	3	6
17	3	3	4	0
19	1	0	2	3

FIG.7B

MOUSE VARIABLE REGION CONSENSUS PRIMERS

MOUSE HEAVY CHAIN J SEGMENTS

JH1 TACTGGTACTTCGATGTCTGGGGCGCAGGGACCAC GGTCAACC GTCTCCTCA
 JH2 TACTTTGACTACTGGGGCCAAGGGACCAC TCTCAG GTCTCCTCA
 JH3 CCTGGTTTGCTTACTGGGGCCAAGGGACTCT GGTCACT GTCTCTGCA
 JH4 TACTATGCTATGGACTACTGGGGTCAAGGAACCTC AGTCACC GTCTCCTCA

CONSENSUS PRIMER: UIG-H AGGGACCAC GGTCAACC GTCTC

BstEII
 TCCCTGGTG CCAGTGG CAGAG
 3' 5'

MOUSE LIGHT CHAIN J SEGMENTS

JK1 TGGACGTTCCGGTGGAGGACC AAGCTG GAAATCAAA
 JK2 TACACGTTCCGAGGGGGGACC AAGCTG GAAATAAAA
 JK4 TTCACGTTCCGGCTCGGGGACA AAGCTG GAAATAAAA
 JK5 CTCACGTTCCGGTGCTGGGACC AAGCTG GAGCTGAAA

CONSENSUS PRIMER: UIG-K GGGACC AAGCTT GAG

HindIII
 CCCTGG TTCGAA CTC
 3' 5'

pGML60

GGAGGGACC AAGGTG GAGATGAAA
 -----C-T-----
 HindIII

FIG.7C

MOUSE $\gamma 2a$ J/C JUNCTION PRIMER

MJ_H2-ApaI TGTCAAGAGGAGTCGGTCTGTGTTCCCGGGTA
 3' ApaI 5'

FIG.7D

206070 010902

HEAVY CHAIN V REGION MODULE GENE SYNTHESIS

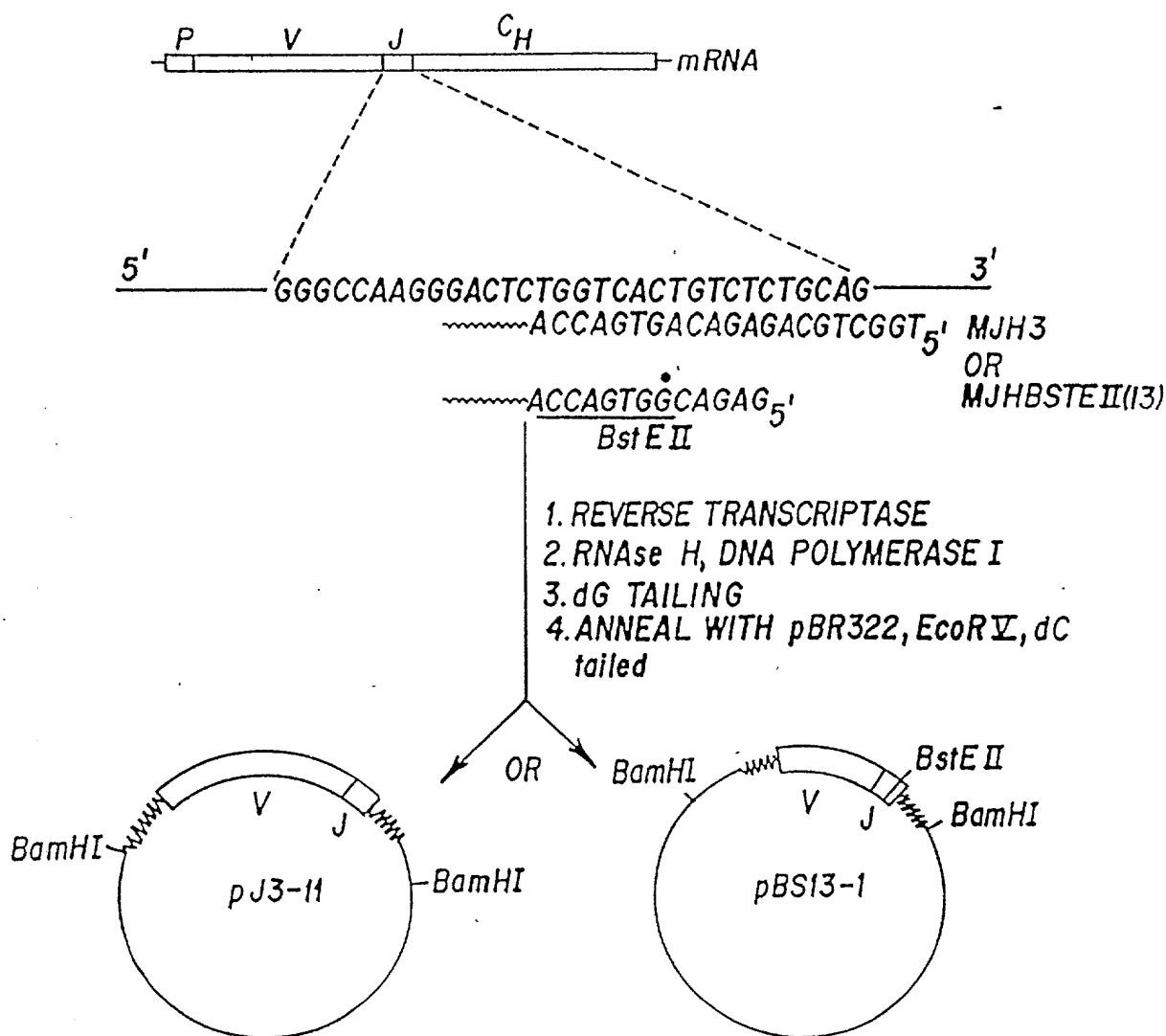


FIG. 8

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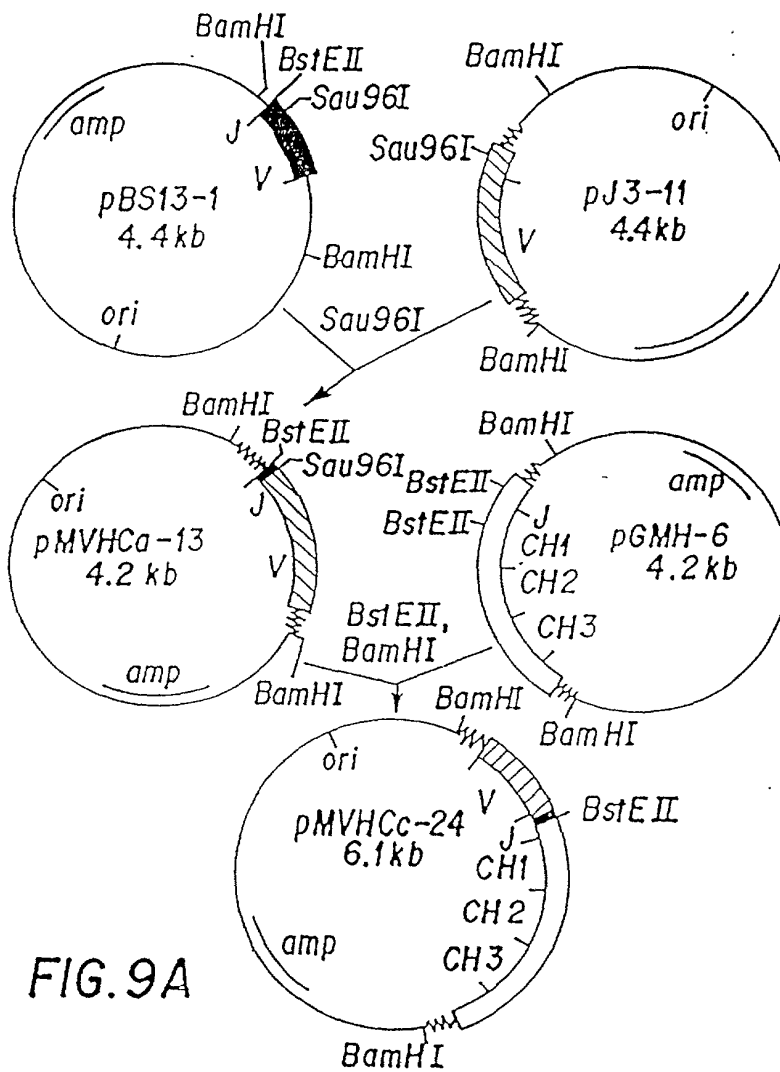


FIG. 9A

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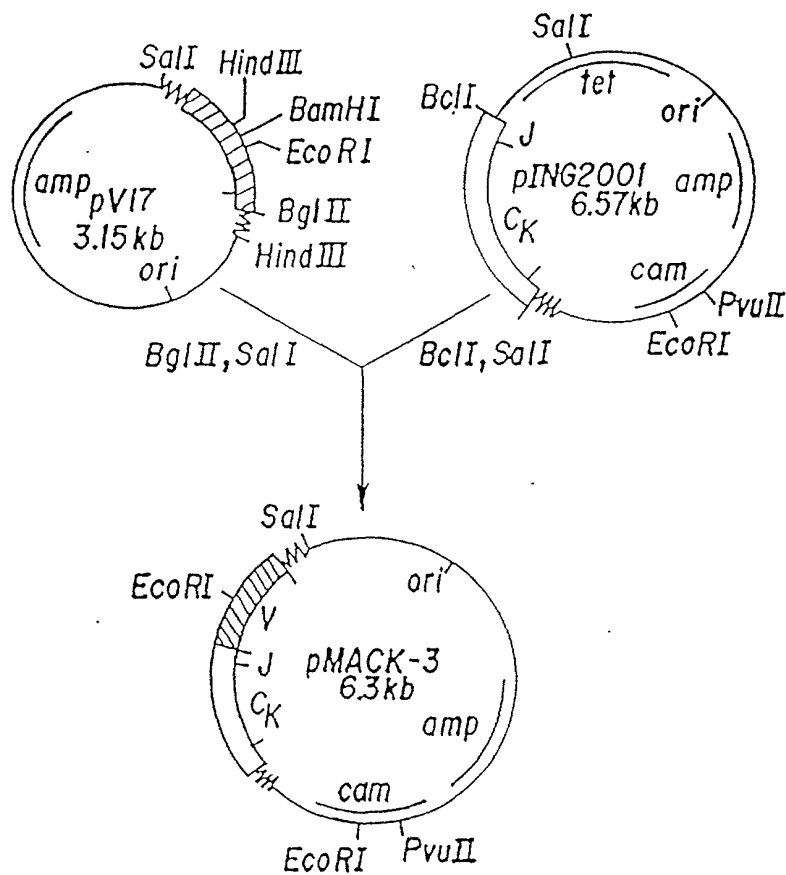


FIG. 9B

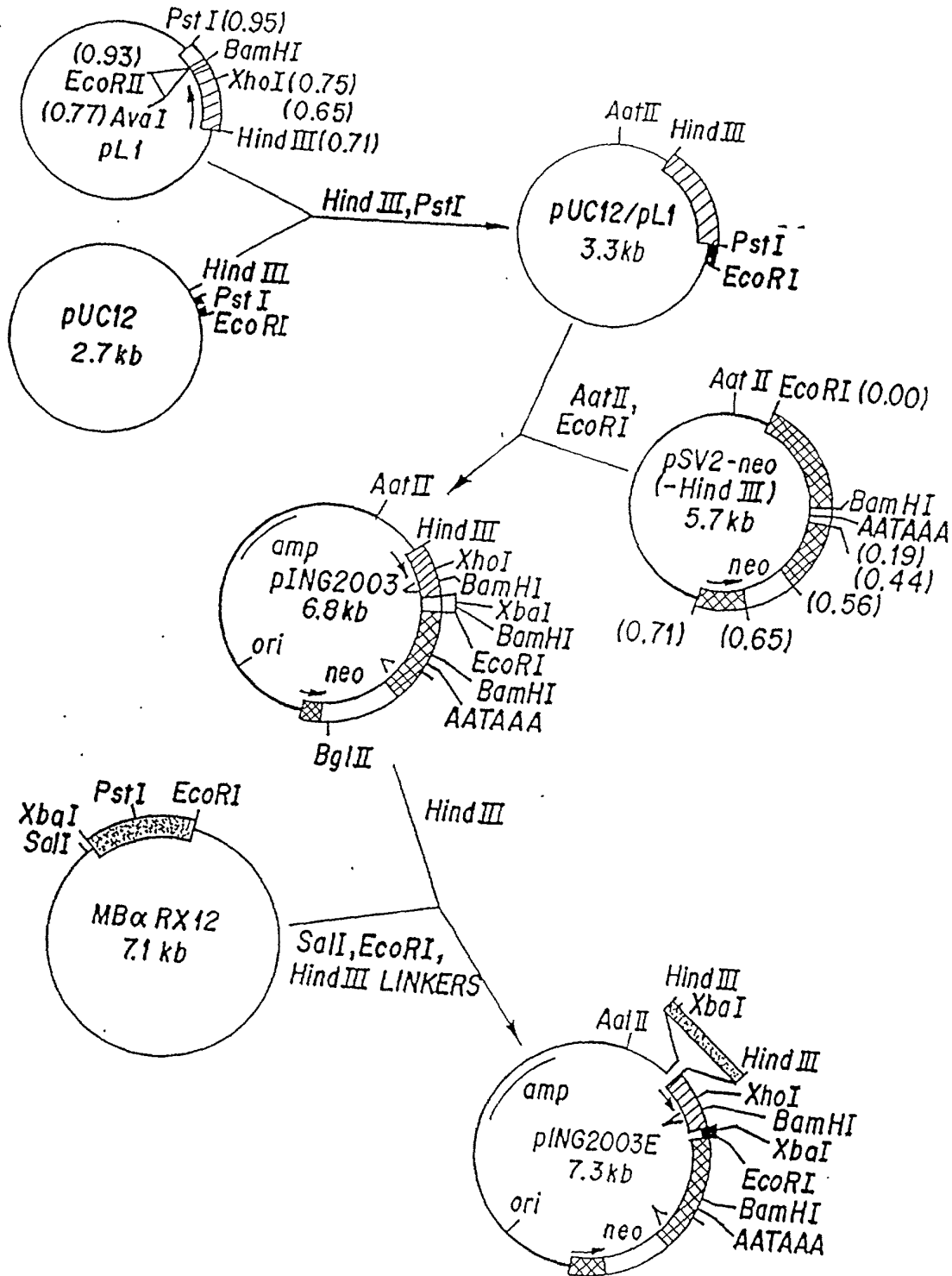


FIG. 10

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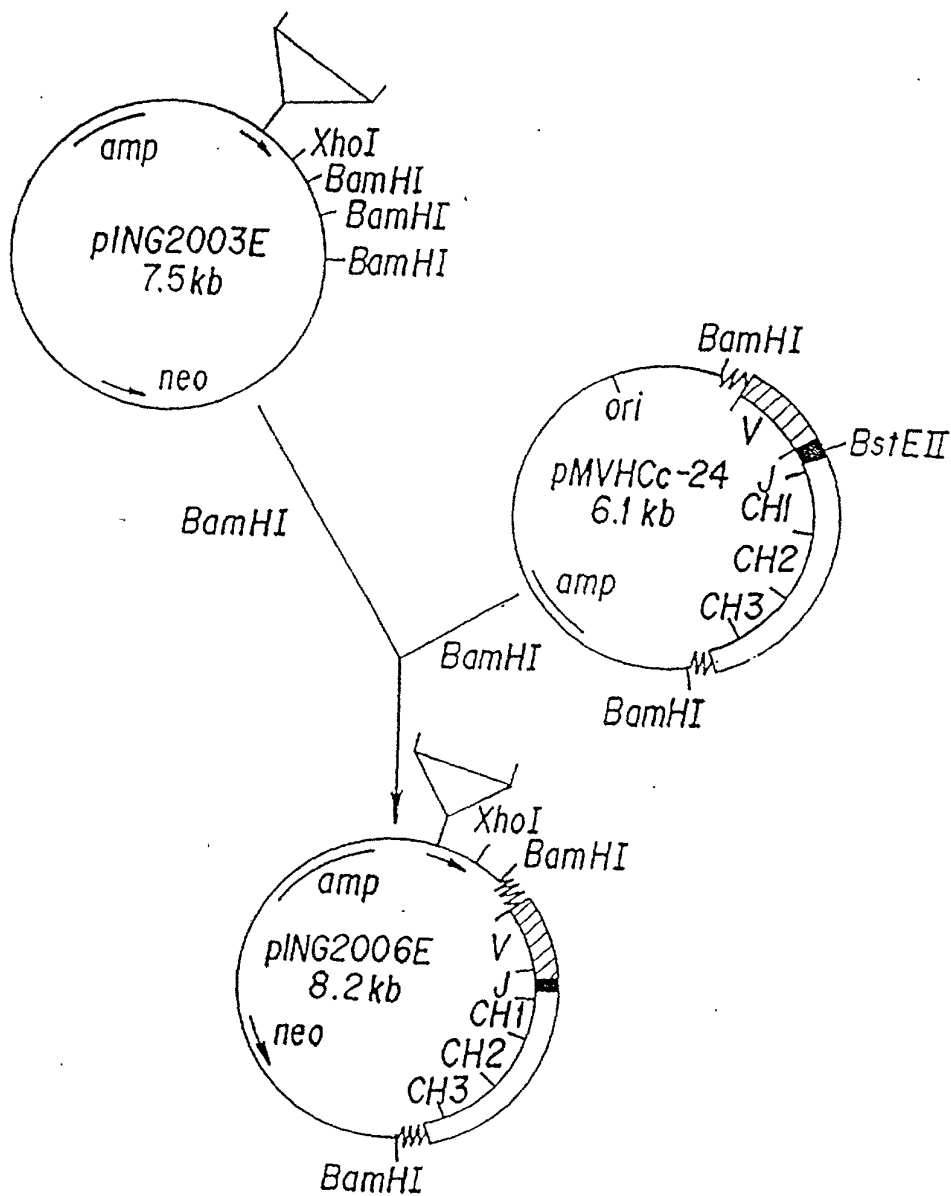


FIG. 11

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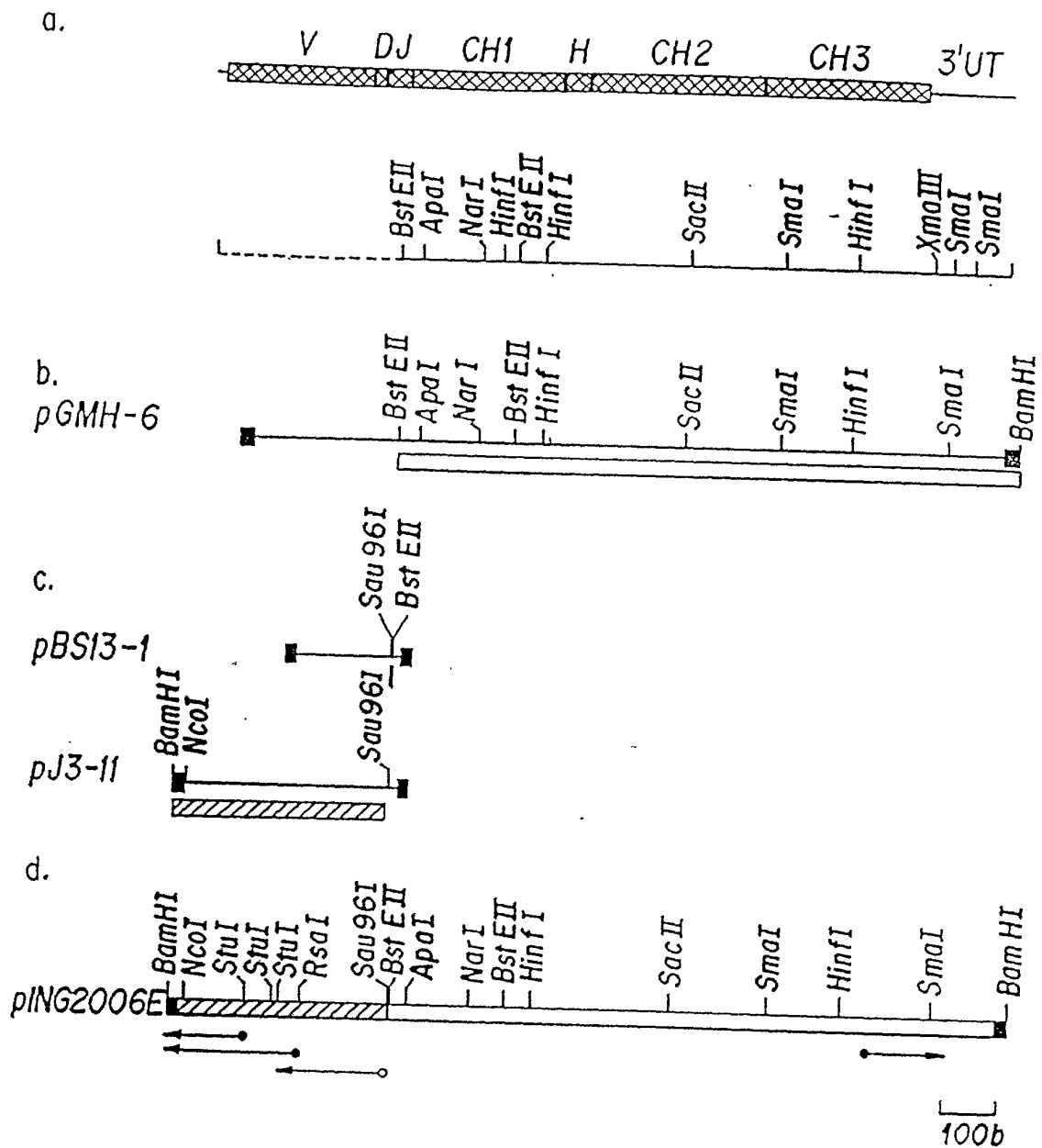


FIG.12A

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206070" 5460400T

PING2006E GGA TCC,CCC ACC ATG MET Gly Trp Ser Tyr Ile Leu Phe Leu Val Ala Thr Ala Arg Asp 60
 PING2012E GGA TCT GTC GAC ATG 2 7 30 45 12 17
 Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala Ser 17
 GTC CAC TCC CAG GTC CAA TTG CAG CAG CCT GCG GCT GAA CTG GTC AAA CCT GGG GCT TCA 120
 Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp MET His Trp Val 37
 GTG AAG GTG TCC TCC TGC AAG GCC TCT GGC TAC ACC TTC ACC AGC TAC TGG ATG CAC TGG GTC 180
 Lys Gln Arg Pro Gly Gln Gly Leu Asp Trp Ile Gly Glu Ile Asn Pro Ser Asn Gly Arg 57
 AAG CAG AGG CCT GGA CAA GGC CTT GAC TGG ATT GGA GAG ATT AAT CCT AGC AAC GGT CGT 240
 Thr Asn Tyr Asn Gln Glu Lys Phe Lys Ser Lys Ala Thr Thr Leu Thr Val Asp Lys Ser Ser 77
 ACT AAT TAC AAT GAG AAG AAG TTC AAG AGC AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC 300
 Thr Ala Tyr MET Gln Leu Ser Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala 97
 ACA GCC TAC ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT GCC 360
 Ser Tyr Asp Tyr Asp Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 117*
 TCC TAT GAT TAC GAC TGG TTT GCT TAC TGG GGC CAA GGC ACT CTG GTC ACC GTC TCC TCA 420

BstEII

FIG.12B

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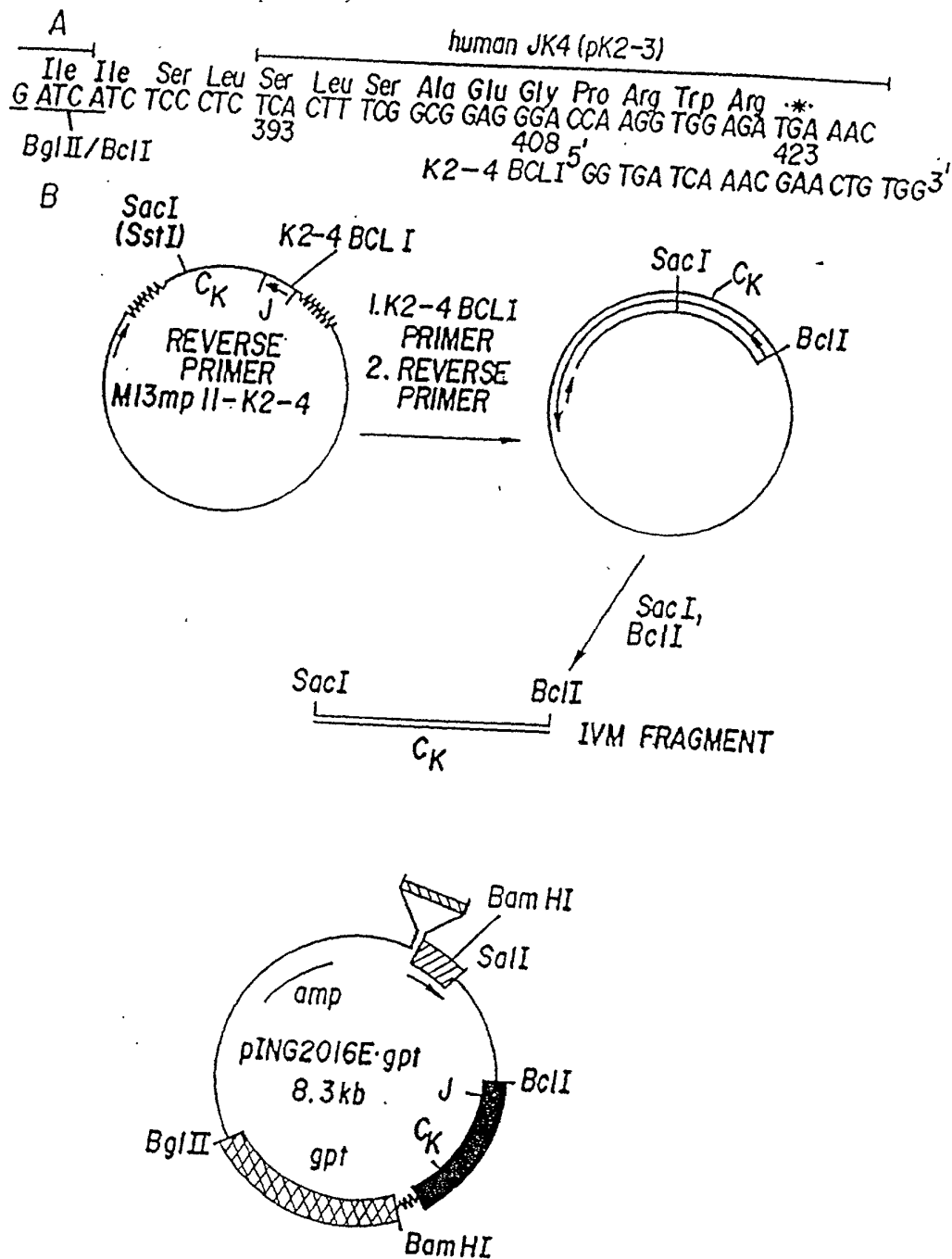


FIG. 13

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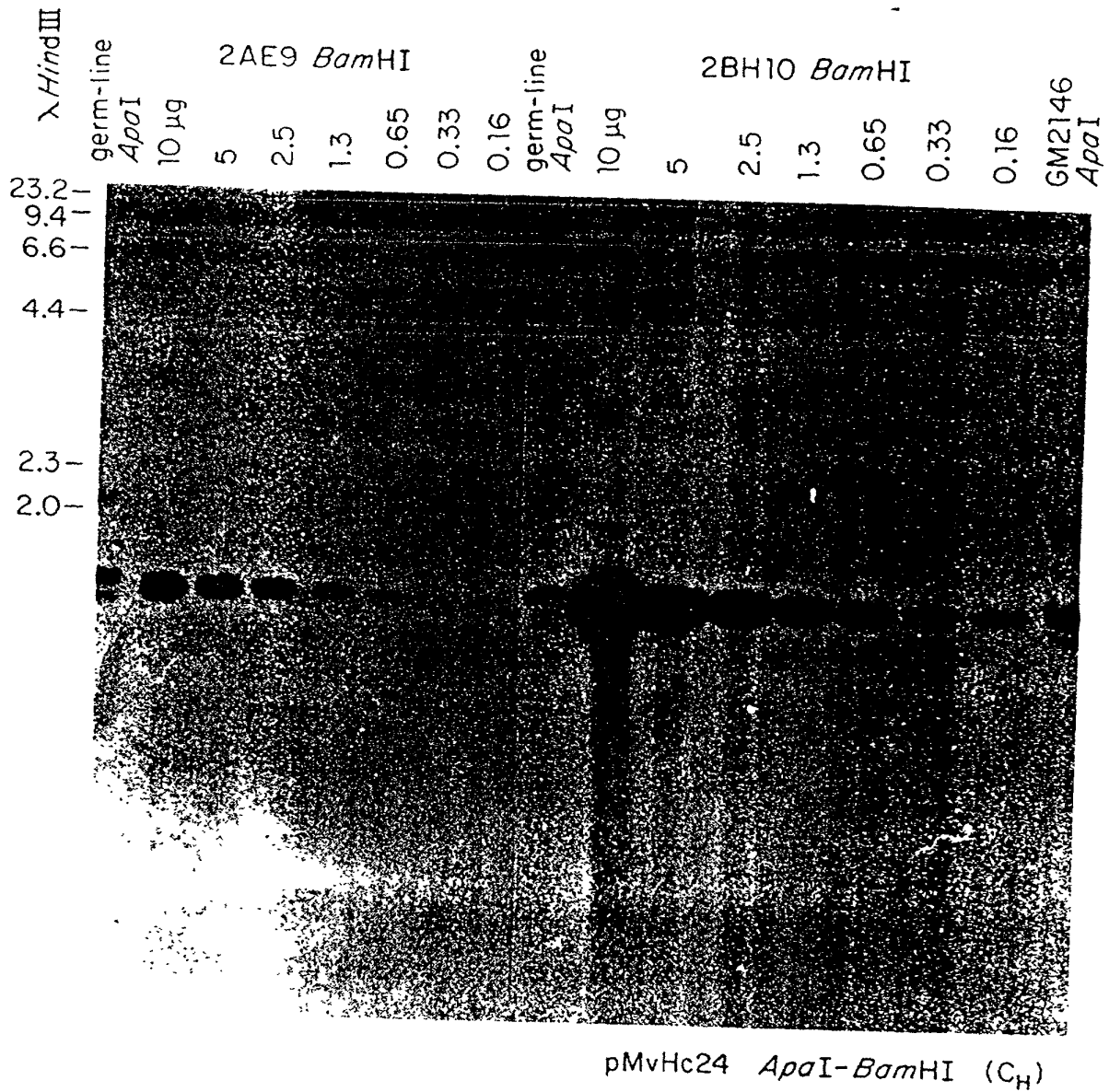


FIG. 14

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206010 "SHOOT"

MET Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser Val Ile MET Ser Arg Gly Gln
 CCC CCC CCC CCC CAA GAC AAA ATG GAT TTT CAA GTG CAG ATT TTC AGC TTC CTG CTA ATC AGT GCT TCA GTC ATA ATG TCC AGA GGA CAA
 15 30 45 60 75 90
 Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Gln Lys Val Thr Leu Thr Cys Arg Ala Ser Ser Ser Val Ser Phe
 ATT GTT CTC TCC TCC CAG TCT CCA GCA ATC CTC TCT GCA TCT CCA GGG GAG GAG AAG GTC ACA TTG ACT TGC AGG GCC AGC TCA AGT GTA AGT TTC
 105 120 135 150 165 180
 CDR1 FRI CDR2 CDR2 FR3
 MET Asn Trp Trp Gln Lys Pro Lys Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Glu Phe Pro Gly Arg Phe
 ATG AAC TGG TAC CAG CAG CAG CCA GGA GGC TCC TCC CCC AAA CCC TCG ATT TAT GCT TCT GAG TTC CCT GGT CGC TTC
 195 210 225 240 255 270
 Ser Gly Glu Trp Ser Gly Thr Ser Tyr Ser Leu Ala Ile Ser Arg Val Glu Ala Glu Asp Ala Thr Tyr Tyr Cys Gln Gln Trp Asn
 AGT GGC GAG TGG TCT GGG ACC TCT TAC TCT CTC GCA ATC AGC AGA GTG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG CAG TGG AAT
 285 300 315 330 345 360
 CDR3 FR4
 Ser Asn Pro Leu Thr Phe Gly Ala Glu Thr Lys Leu Glu Lys Lys Arg
 AGT AAC CCA CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGG
 375 390 405

— T — Hind III PRIMER

V_K J_K5 C_K An

BamHI HpaI

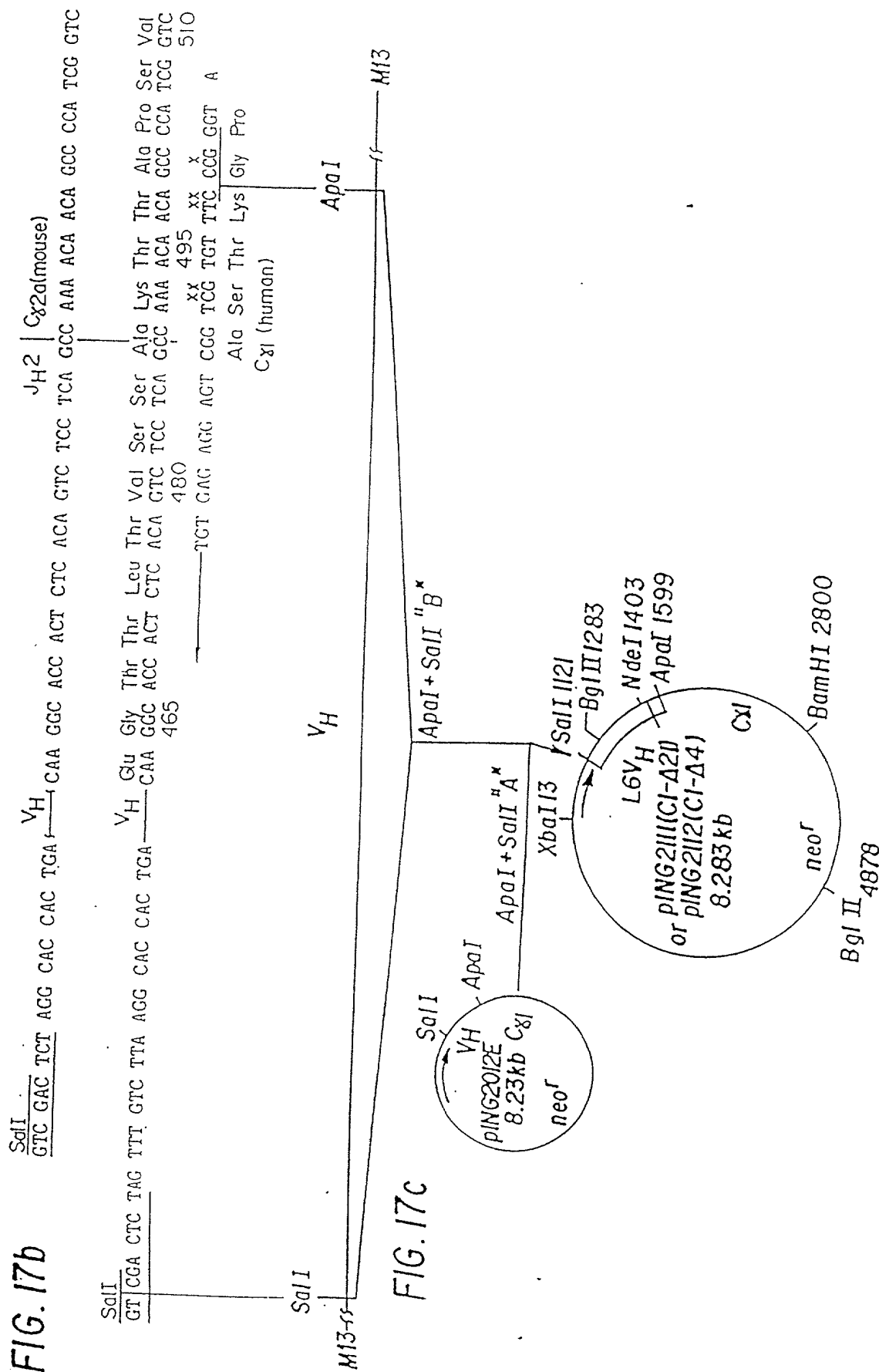
pL3-12a

100bp

FIG.16

FIG. 17a

101	101
102	102
103	103
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199	199
200	200



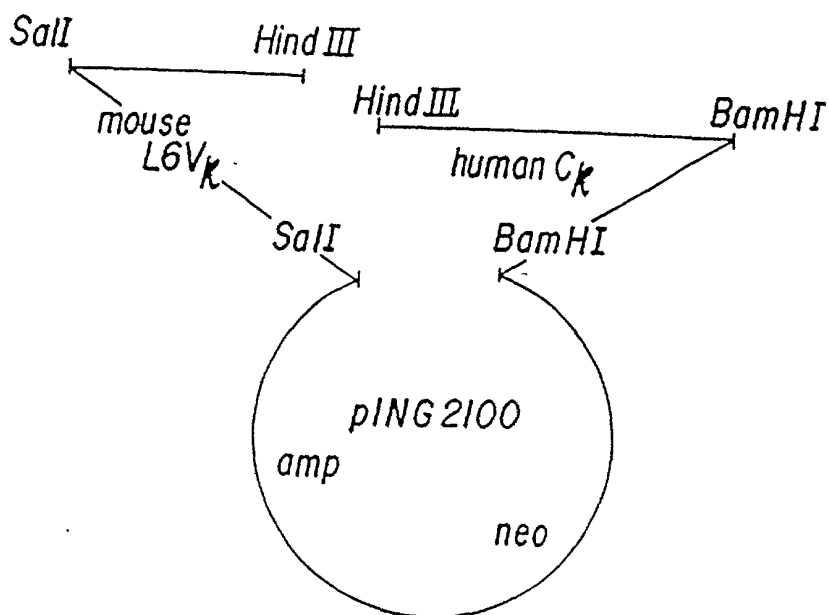


FIG.18

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5' L6V_k 'met asp phe
 -CCCCAAGACAAAATGGATTTTC-
 III
 GTC

 Sal I

FIG.19A

20040915 040902

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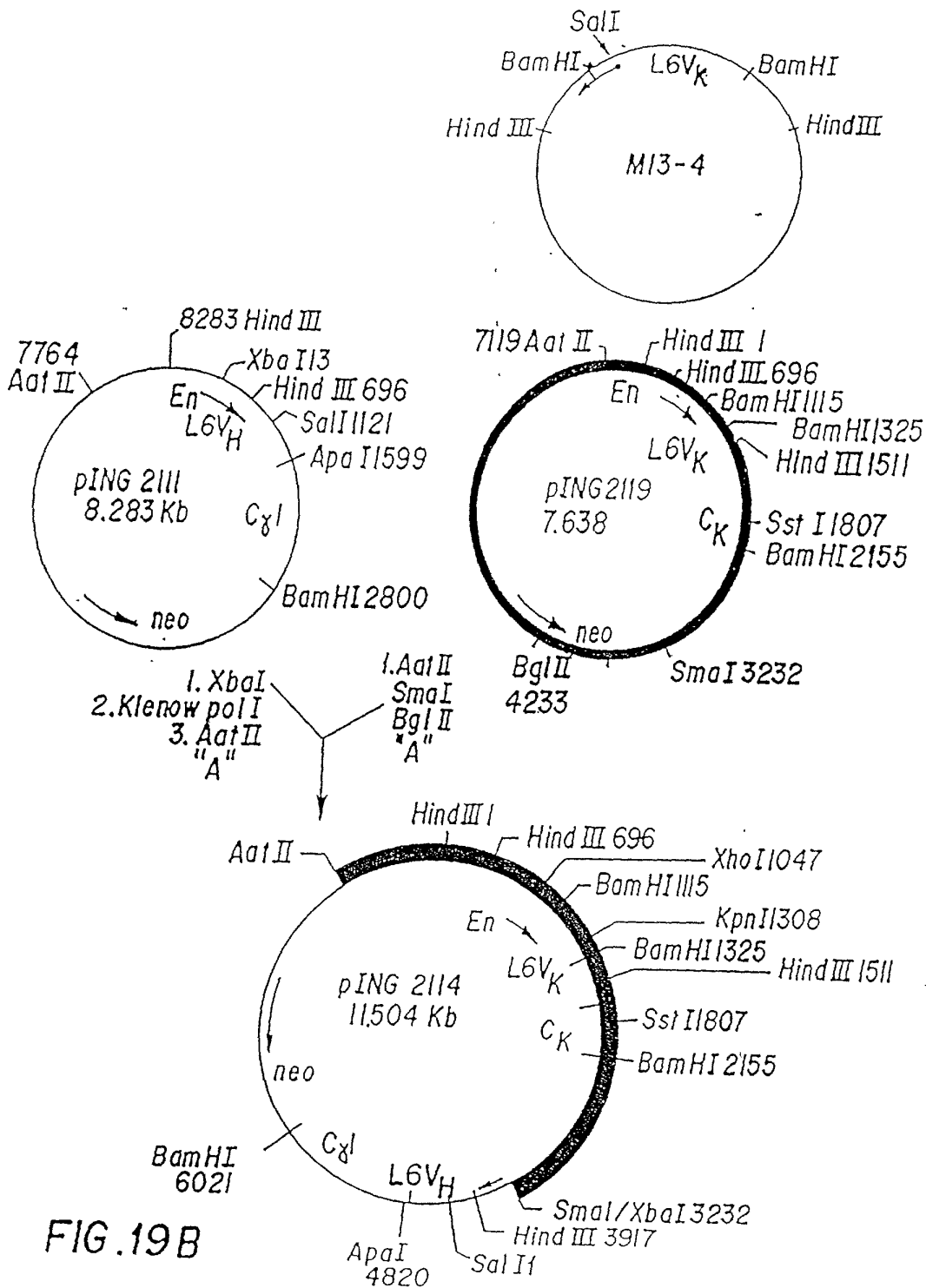


FIG. 19B

206070-5464007

L6 Chimerae

V_H pH3-6A (J_H2) oligo (dT) clone, BAL -31 detections 5' CxIAPA mutagenesis → pING 2111 neo
pING 2112 neo

Sall ↓ pING2111 pING2112

5' GTCGACTCTAGTTTGTCTTTAAGGCACCACTGAGCCCCAAG

met
TCTTAGACATCATGGAT

joint ACCACTCTCACA GTC TCC TCA | GCC AGC ACA AAG GGC
mo | hu
Apal ↓
C CAT

V_K p13-12A (J_K5) oligo (dT) clone. J_K Hind III mutagenesis, 5' SAL mutagenesis → pING 2119 neo
pING 2120 got

Sall ↓ met

5' GTCGACAAAATG GAT

joint ACC AAG CT (T) GAG (A) TG AAA | CGA ACT
mo | hu
 C_K

FIG. 20

leader

C₃₃GTACCTCTCTACAGTCCCTGAAGACACTGACTCTAACCATG GGA TTC AGC AGG ATC TTT
peptide NcoI FRI

leu phe leu leu ser val thr thr gly val his ser gln ala tyr leu gln
CTC TTC CTC CTG TCA GTA ACT ACA GGT GTC CAC TCC CAG GCT TAT CTA CAG

gln ser gly ala glu leu val arg pro gly ala ser val lys met ser cys
CAG TCT GGG GCT GAG CTG GTG AGG CCT GGG GCC TCA GTG AAG ATG TCC TGC

FRI CDR1 CDR1 FR2

lys ala ser gly tyr thr phe thr ser tyr asn met his trp val lys gln
AAG GCT TCT GGC TAC ACA TTT ACC AGT TAC AAT ATG CAC TGG GTA AAG CAG

FR2 CDR2

thr pro arg gln gly leu glu trp ile gly ala ile tyr pro gly asn gly
ACA CCT AGA CAG GGC CTG GAA TGG ATT GGA GCT ATT TAT CCA GGA AAT GGT

CDR2 FR3

asp thr ser tyr asn gln lys phe lys gly lys ala thr leu thr val asp
GAT ACT TCC TAC AAT CAG AAG TTC AAG GGC AAG GCC ACA CTG ACT GTA GAC

lys ser ser ser thr ala tyr met gln leu ser ser leu thr ser glu asp
AAA TCC TCC AGC ACA GCC TAC ATG CAG CTC AGC AGC CTG ACA TCT GAA GAC

FR3 CDR3

ser ala val tyr phe cys ala arg val val tyr tyr ser asn ser tyr trp
TCT GCG GTC TAT TTC TGT GCA AGA GTG GTG TAC TAT AGT AAC TCT TAC TGG

CDR3 FR4 J_H1 FR4 DSP2

tyr phe asp val trp gly thr gly thr thr val thr val ser
TAC TTC GAT GTC TGG GGC ACA GGG ACC ACG GTC ACC GTC TCG

30

BstEII J_HBstEII primer

FIG. 21

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2H7 light chain variable sequence

leader peptide

C₂₃ CCCAAAATTCAAAGACAAAATG GAT TTT CAA GTG CAG ATT TTC AGC TTC CTG CTA
 GTC SalI primer FRI

ile ser ala ser val ile ile ala arg gly¹ gln ile val leu ser gln ser
 ATC AGT GCT TCA GTC ATA ATT GCC AGA GGA CAA ATT GTT CTC TCC CAG TCT

pro ala ile leu ser ala ser pro gly glu lys val thr met thr cys arg^{FRI}
 CCA GCA ATC CTG TCT GCA TCT CCA GGG GAG AAG GTC ACA ATG ACT TGC AGG

CDR1
 ala ser ser ser val ser tyr met his^{CDR1} trp^{FR2} tyr gln gln lys pro gly ser
 GCC AGC TCA AGT GTA AGT TAC ATG CAC TGG TAC CAG CAG AAG CCA GGA TCC

ser pro lys pro trp ile tyr^{FR2} ala pro ser asn leu ala ser^{CDR2} gly^{FR3} val pro^{BamHI}
 TCC CCC AAA CCC TGG ATT TAT GCC CCA TCC AAC CTG GCT TCT GGA GTC CCT

ala arg phe ser gly ser gly ser gly thr ser tyr ser leu thr ile ser
 GCT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATC AGC

arg val glu ala glu asp ala ala thr tyr tyr cys^{FR3} gln gln trp ser phe
 AGA GTG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG CAG TGG AGT TTT

asn pro pro thr phe gly ala gly thr lys leu glu leu lys^{FR4}
 AAC CCA CCC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA

J_K HIND III primer

FIG. 22

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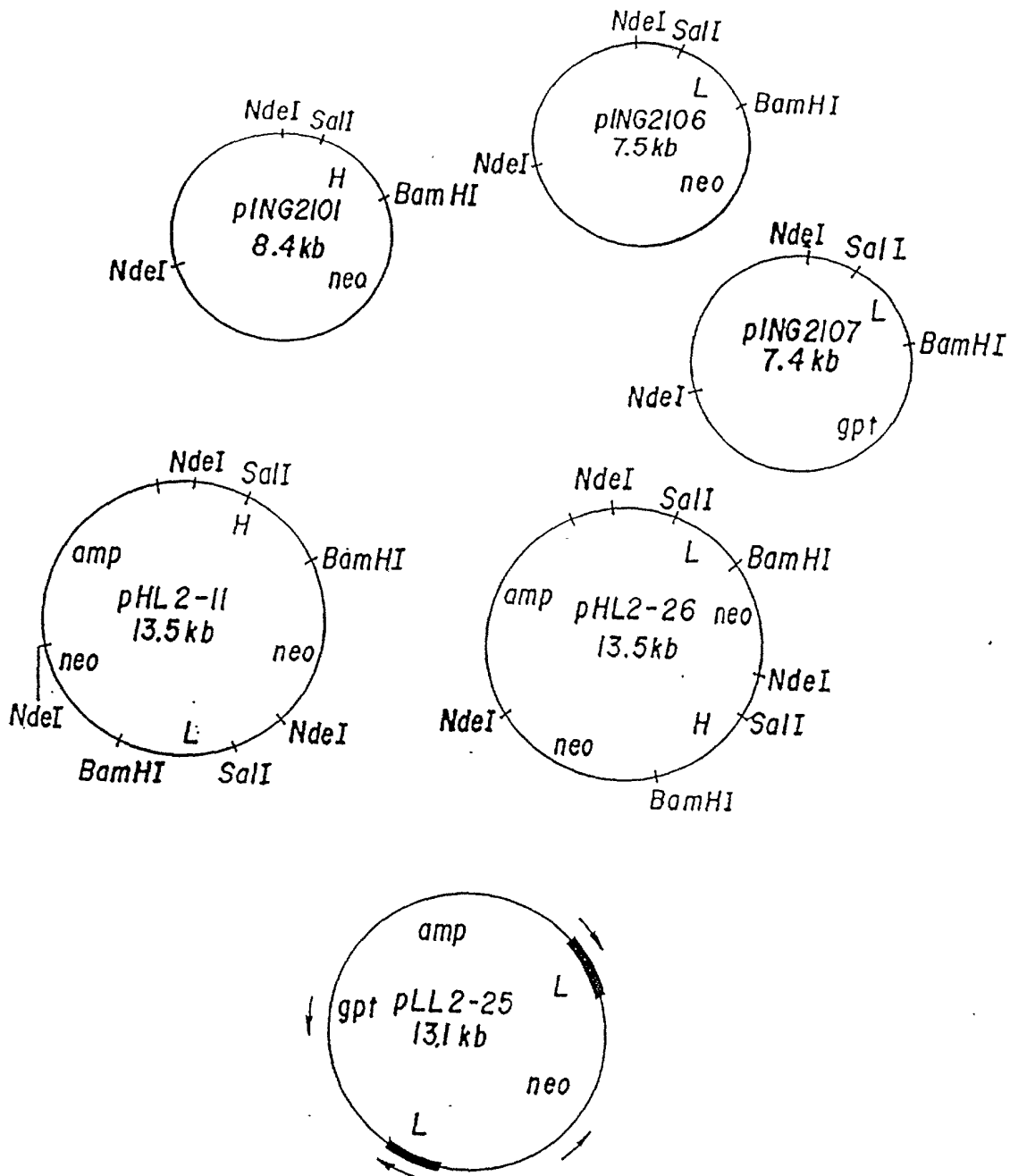


FIG. 23

2H7 Chimerae

V_H PH2-7 (J_H1) J_H BstEII clone, NcoI cut 5' ATG — pING2101 neo

5' $\xrightarrow{\text{SalI}}$ $\xrightarrow{\text{met}}$ GTC GACATGGGA

joint $\xrightarrow{\text{mo, hu}}$ ACGGTC ACC GTC $\text{TC} \textcircled{\text{T}}$ TCA $\xrightarrow{\text{C} \times \text{I}}$ GCC TCC

V_K PL2-12 (J_K5) oligo(dT) clone, J_K HindIII mutagenesis, 5' SAL mutagenesis \rightarrow pING2106 neo
pING2107 gpt

5' $\xrightarrow{\text{SalI}}$ $\xrightarrow{\text{met}}$ GTC GAC AAA ATG GAT

joint $\xrightarrow{\text{neo, hu}}$ ACC AAG $\text{CT} \textcircled{\text{T}}$ GAG $\text{A} \textcircled{\text{T}}$ G AAA $\xrightarrow{\text{C} \times \text{I}}$ CGA ACT

FIG. 24

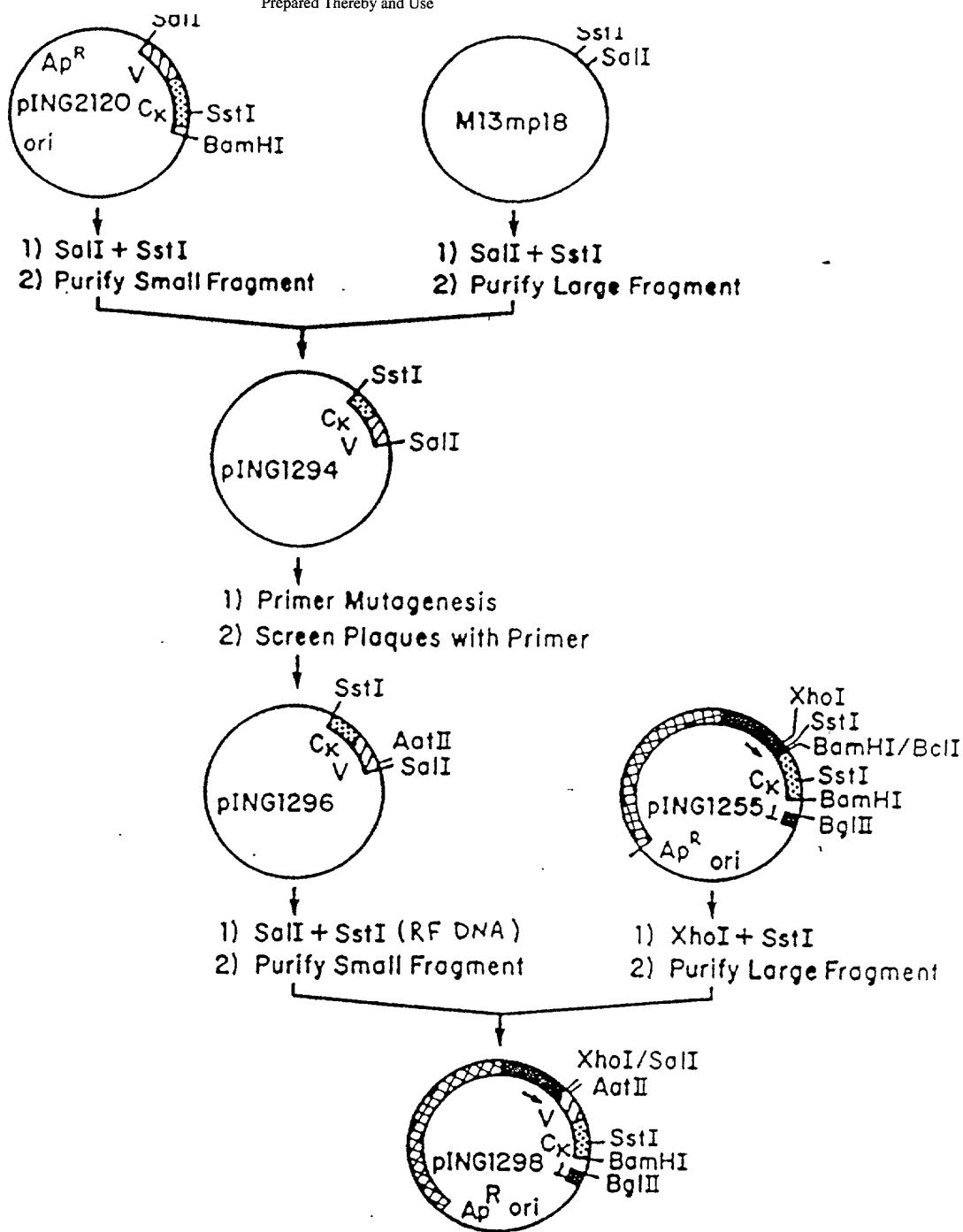


FIG.25A

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 Inventor(s): Robinson et al.; Tel: 202/371-2600
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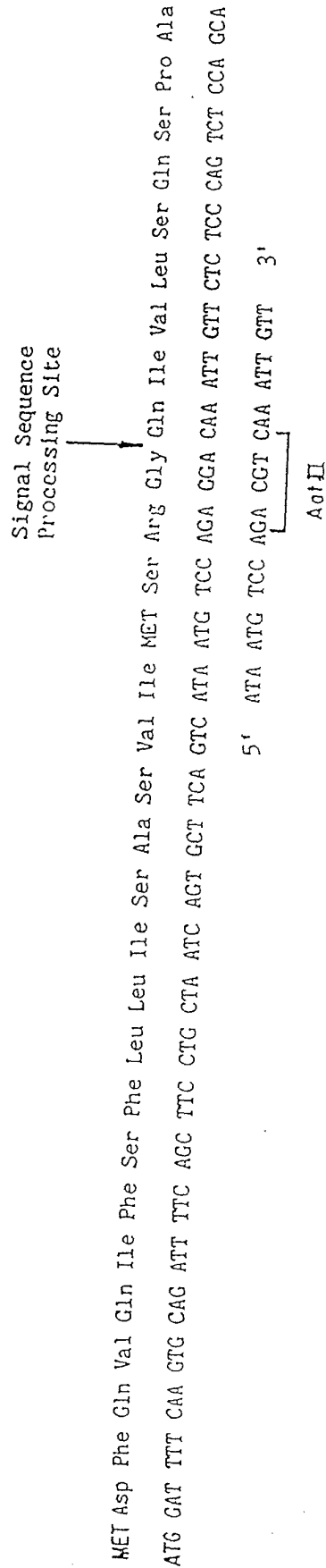


FIG. 25B

Appl. No. To Be Assigned; Group Art Unit: To Be Assigned
 Dkt. No. 0610.0050001/MAC;
 Inventor(s): Robinson et al.; Tel: 202/371-2600
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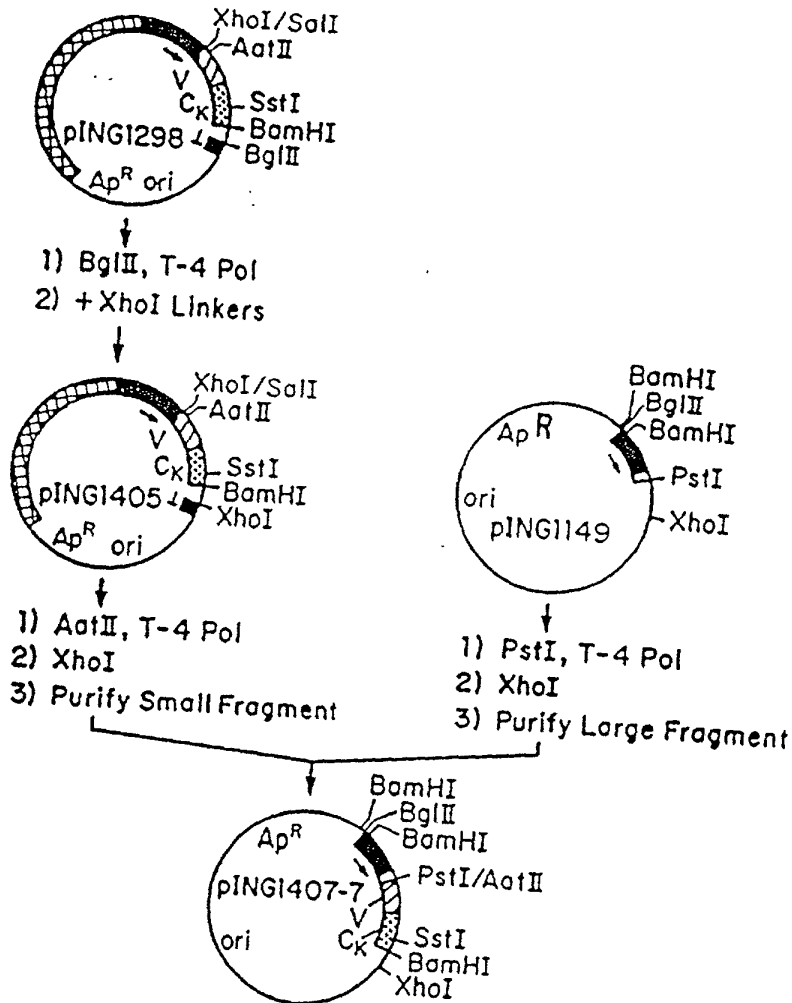


FIG.25C

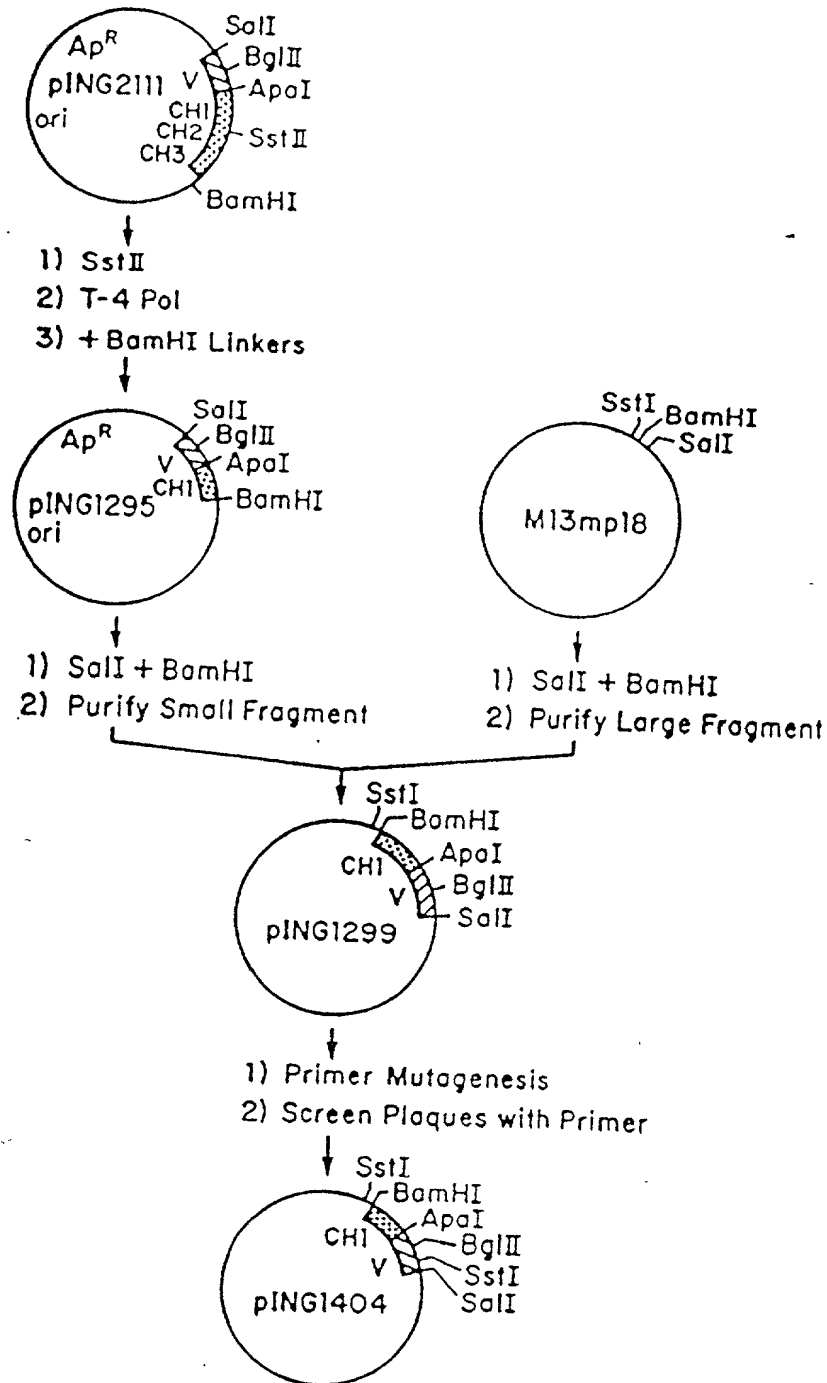


FIG. 26A

Appl. No. To Be Assigned; Group Art Unit: To Be Assigned
 Dkt. No. 0610.0050001/MAC;
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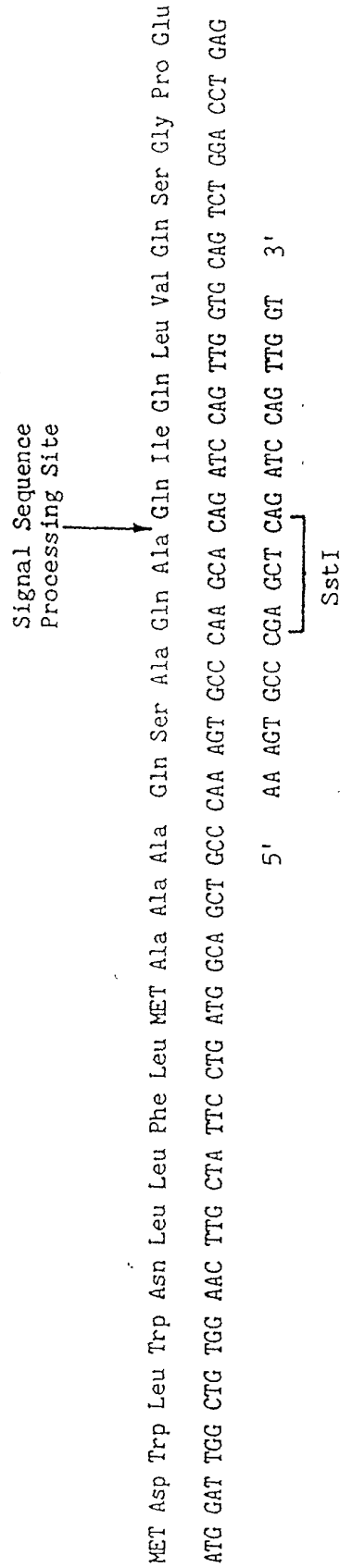


FIG.26 B

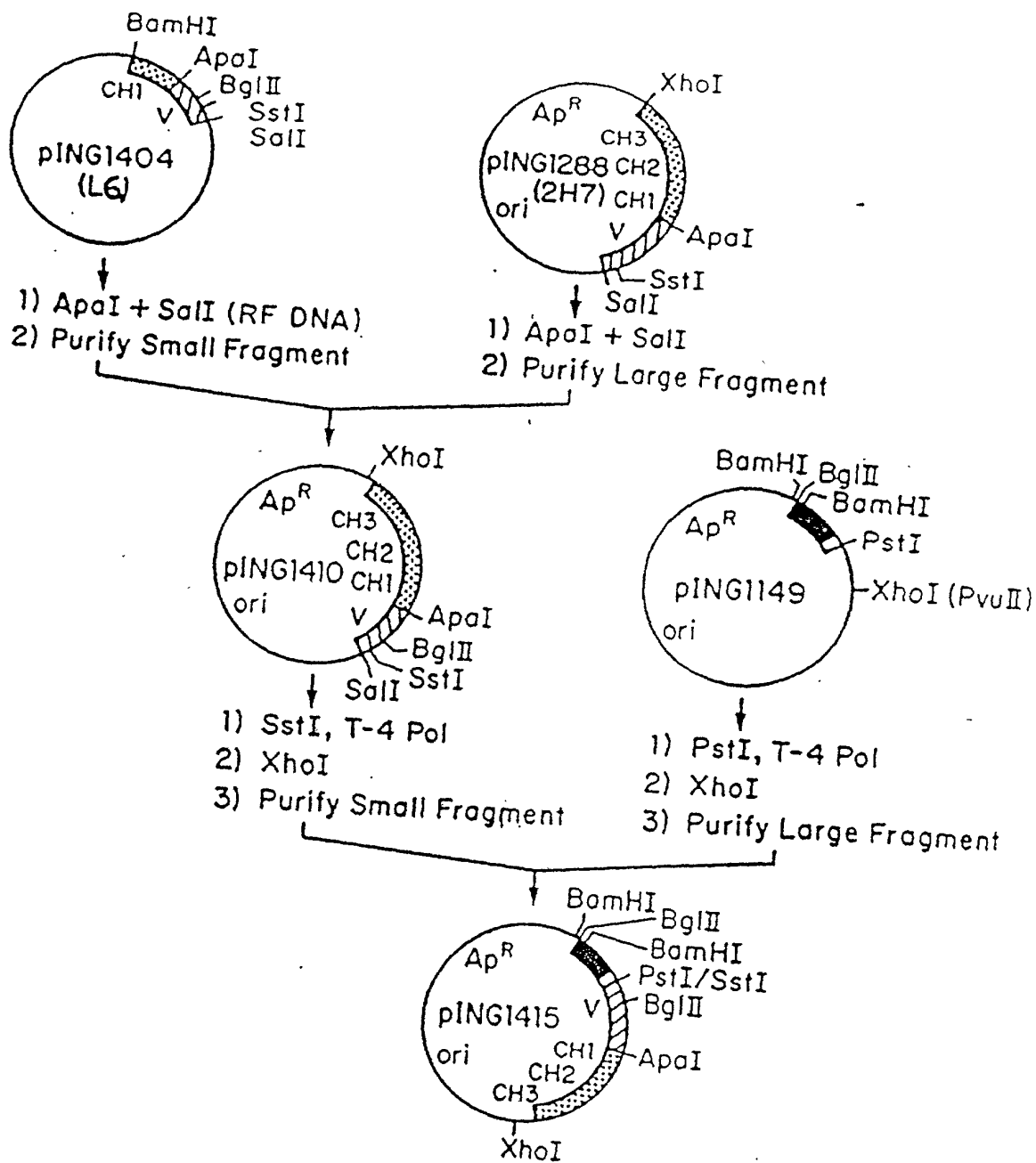


FIG.26C

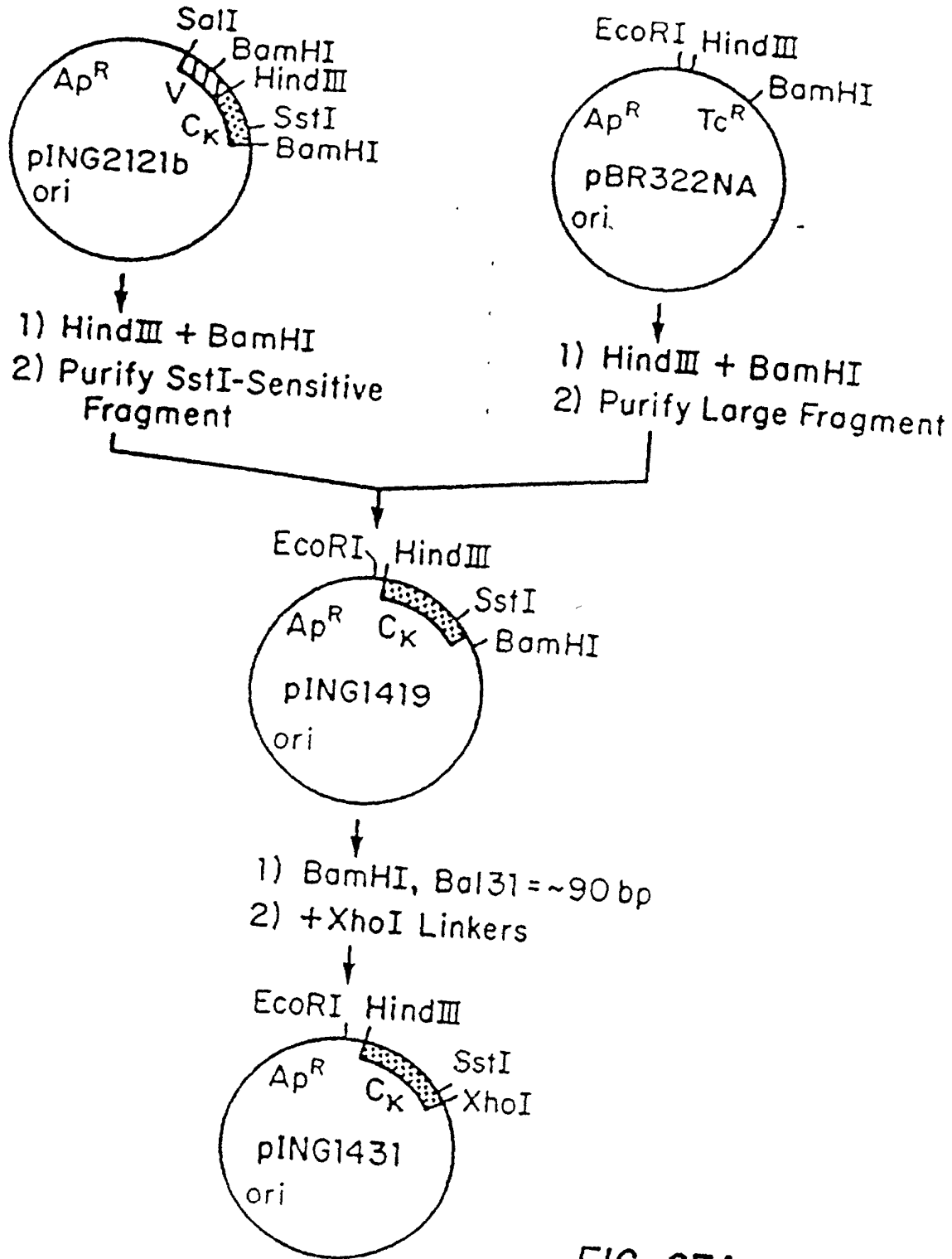


FIG. 27A

206070 " 5460400T

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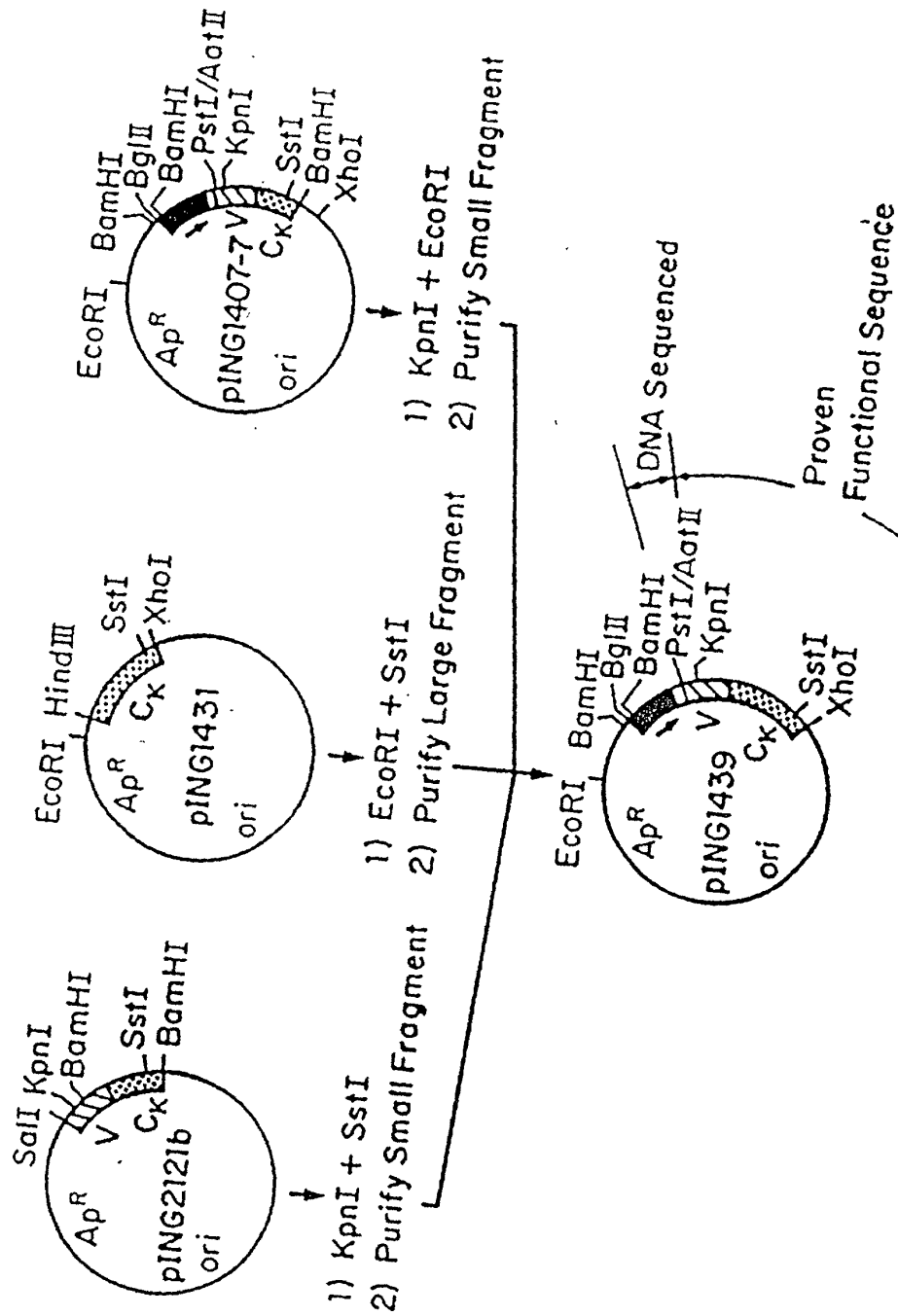


FIG. 27B

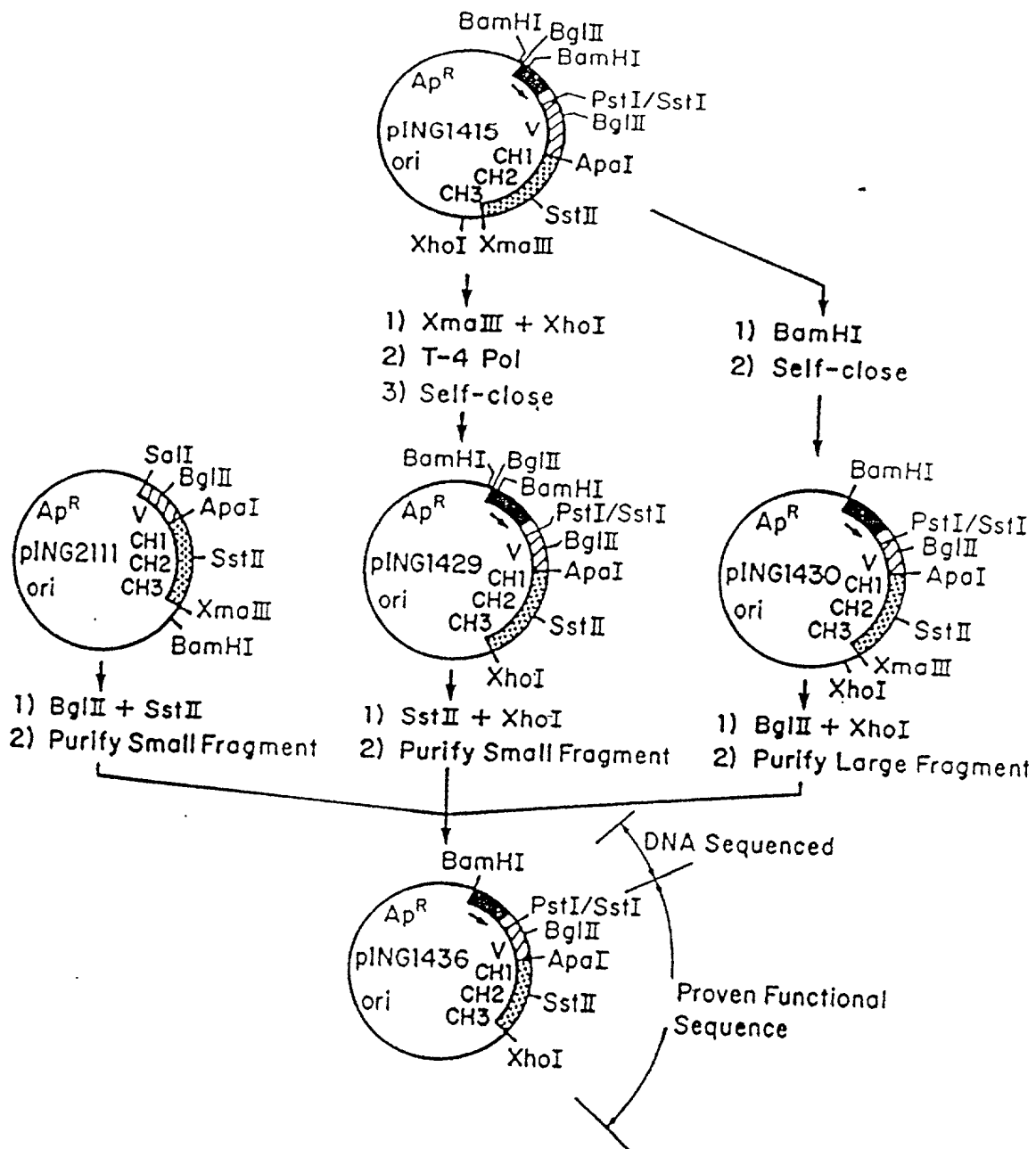


FIG.28

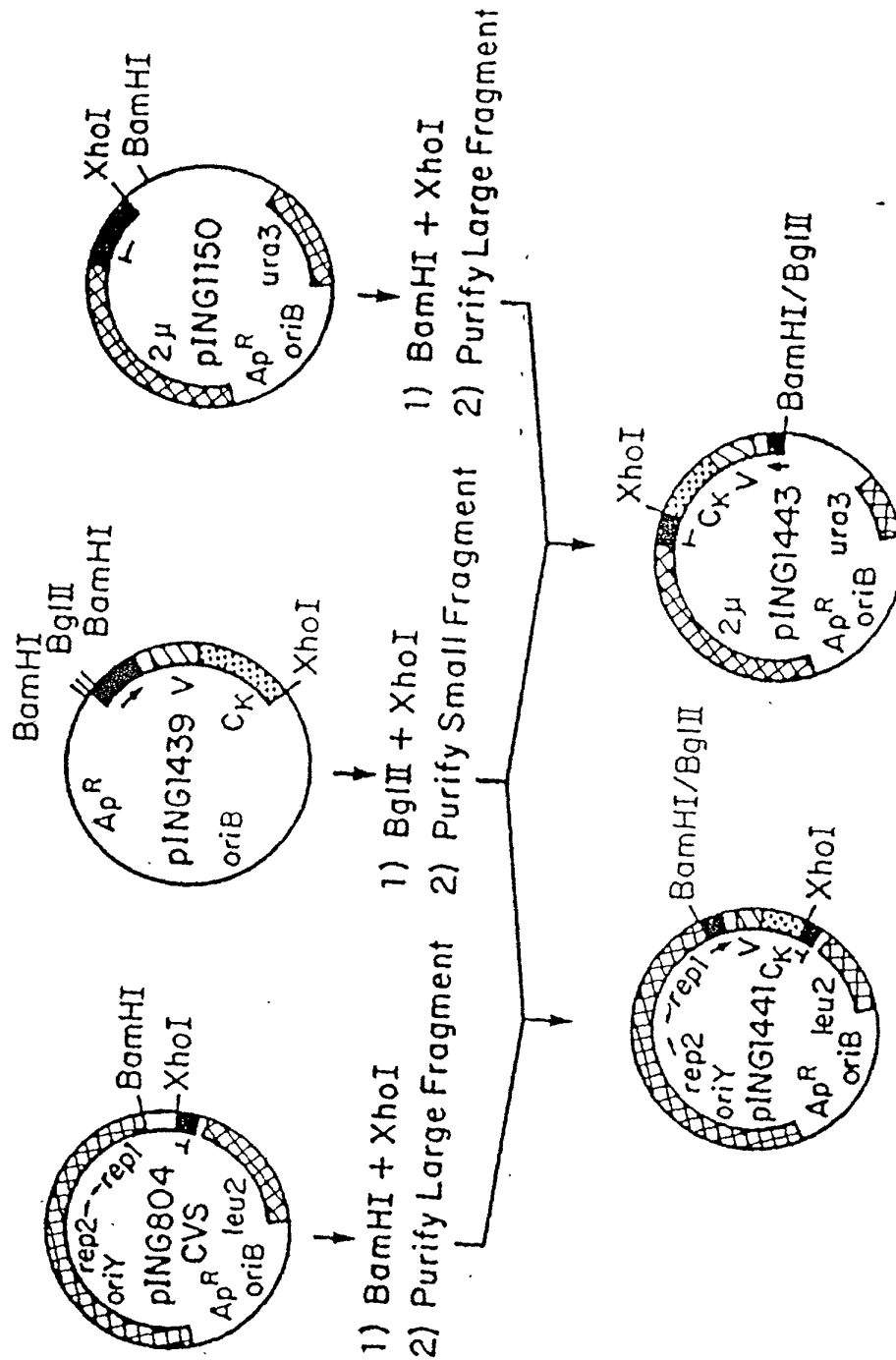


FIG. 29

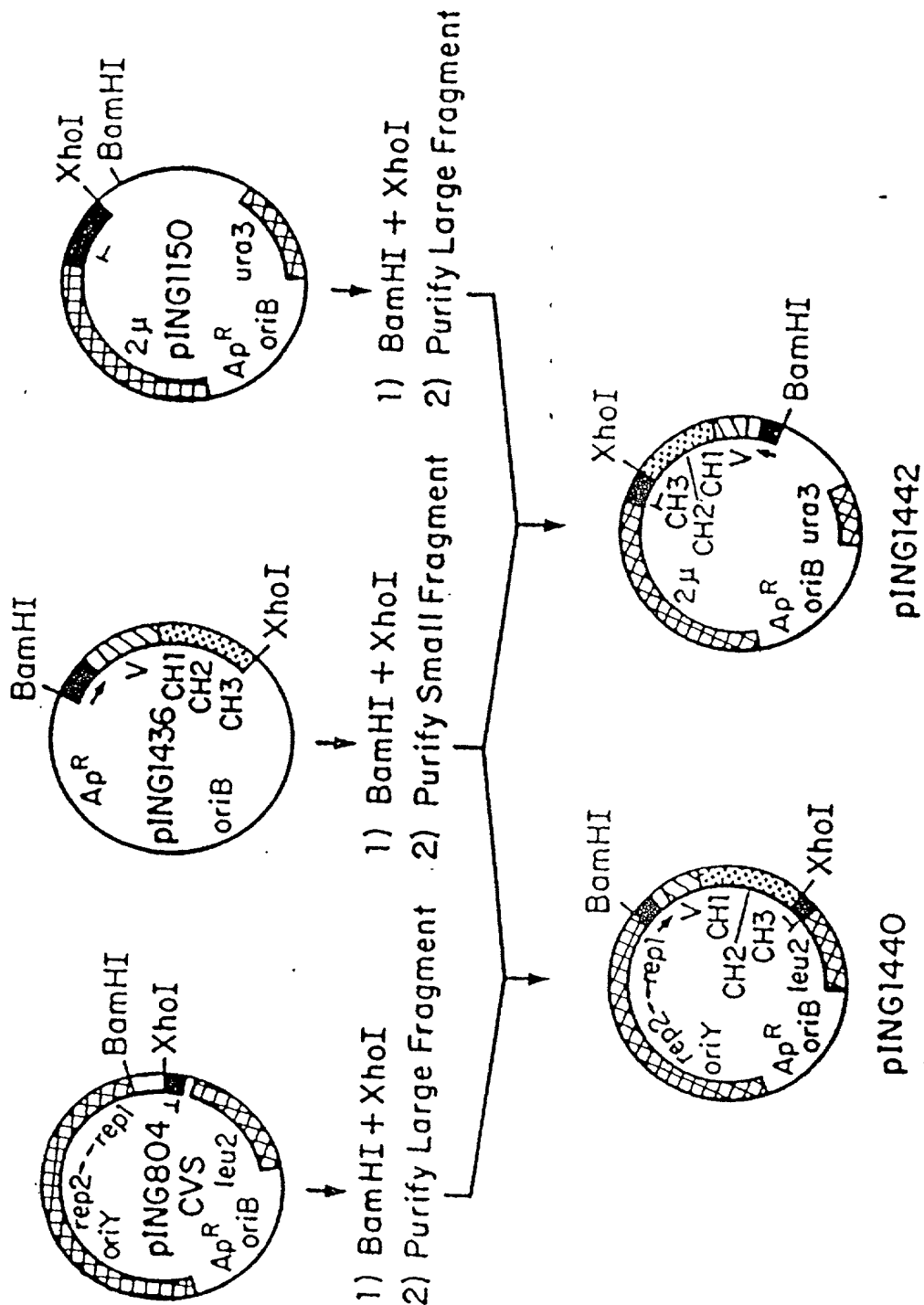
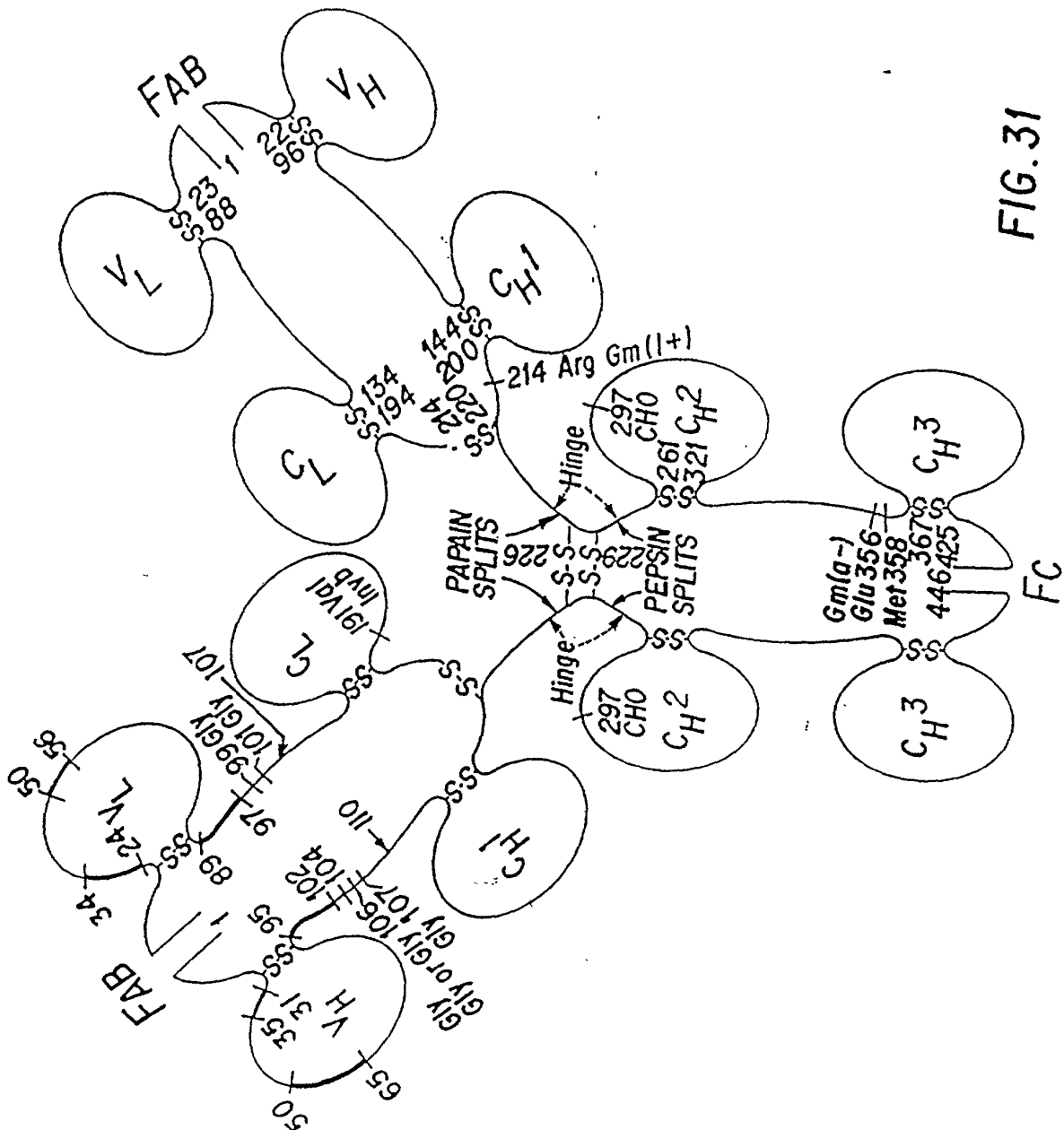


FIG. 30



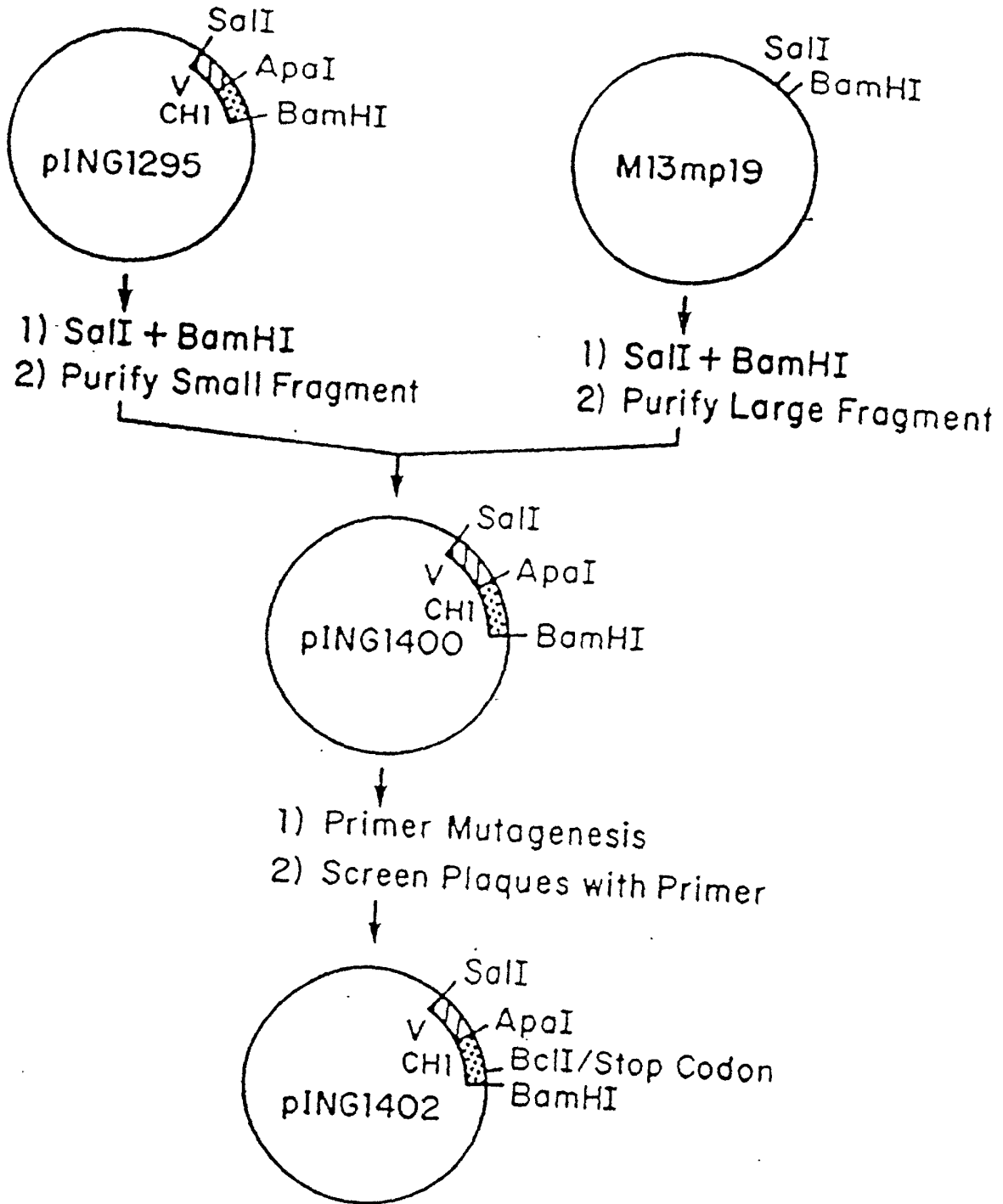


FIG. 32A

Appl. No. To Be Assigned, Group Art Unit: To Be Assigned
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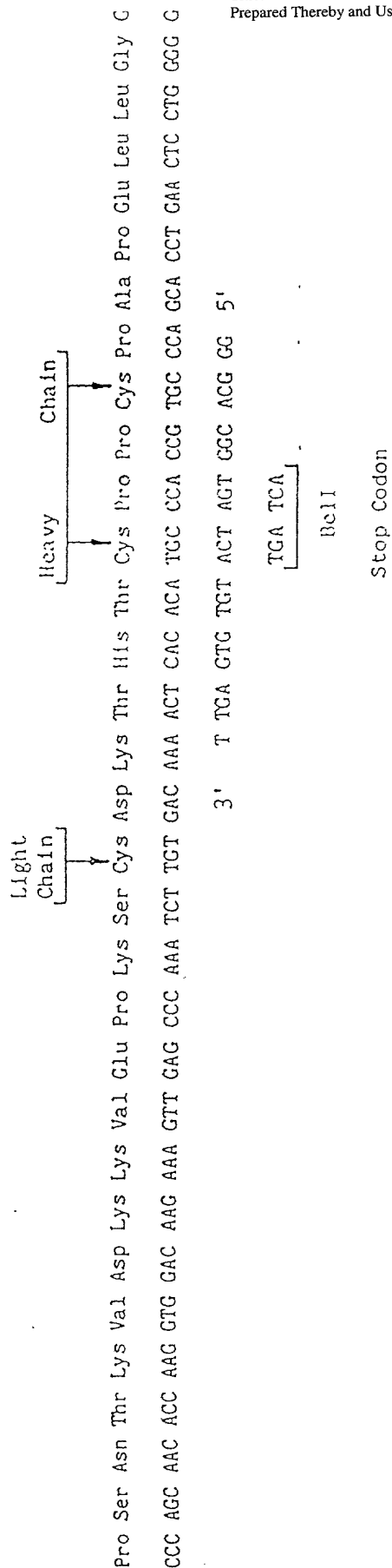


FIG. 32B

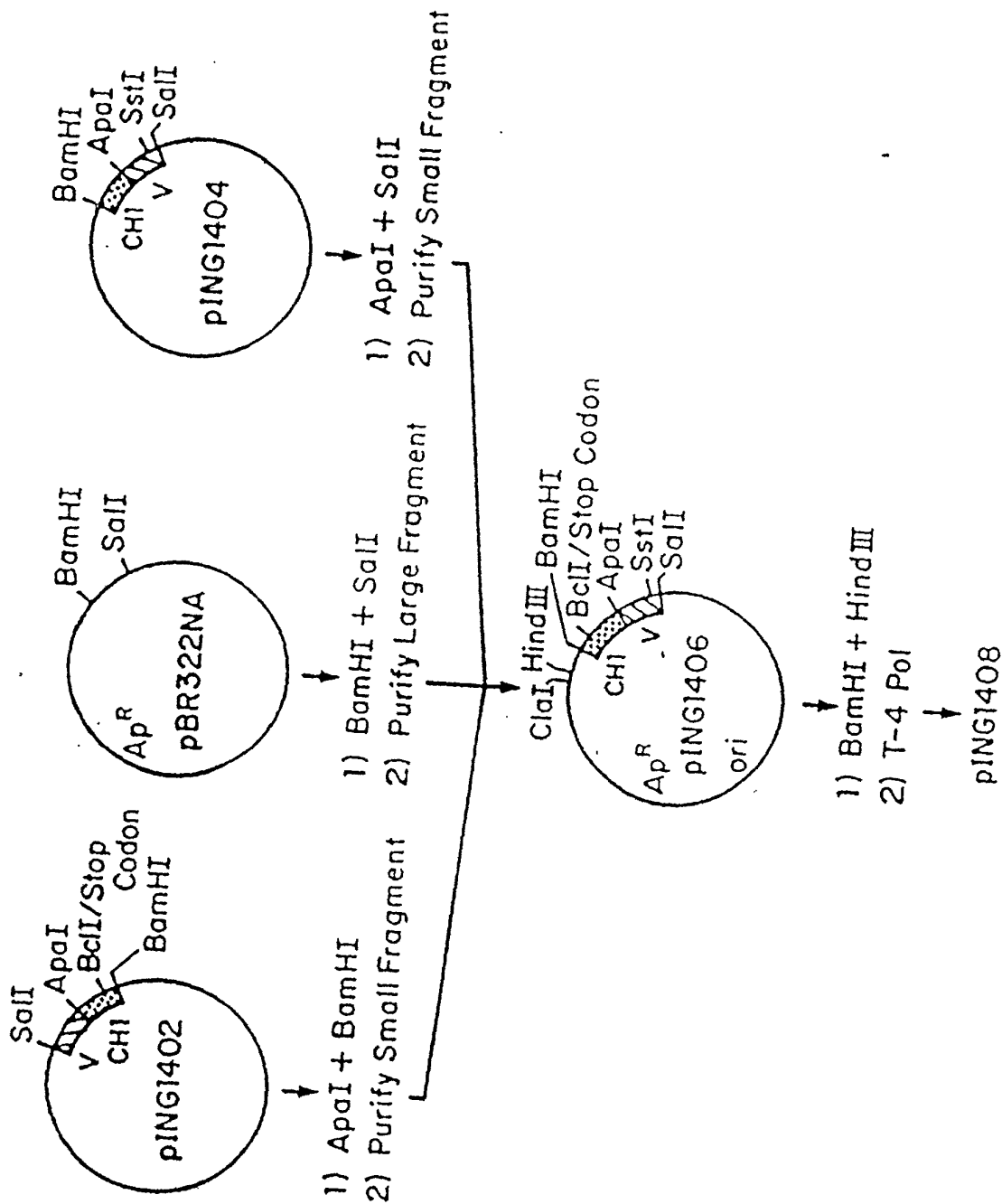


FIG. 33A

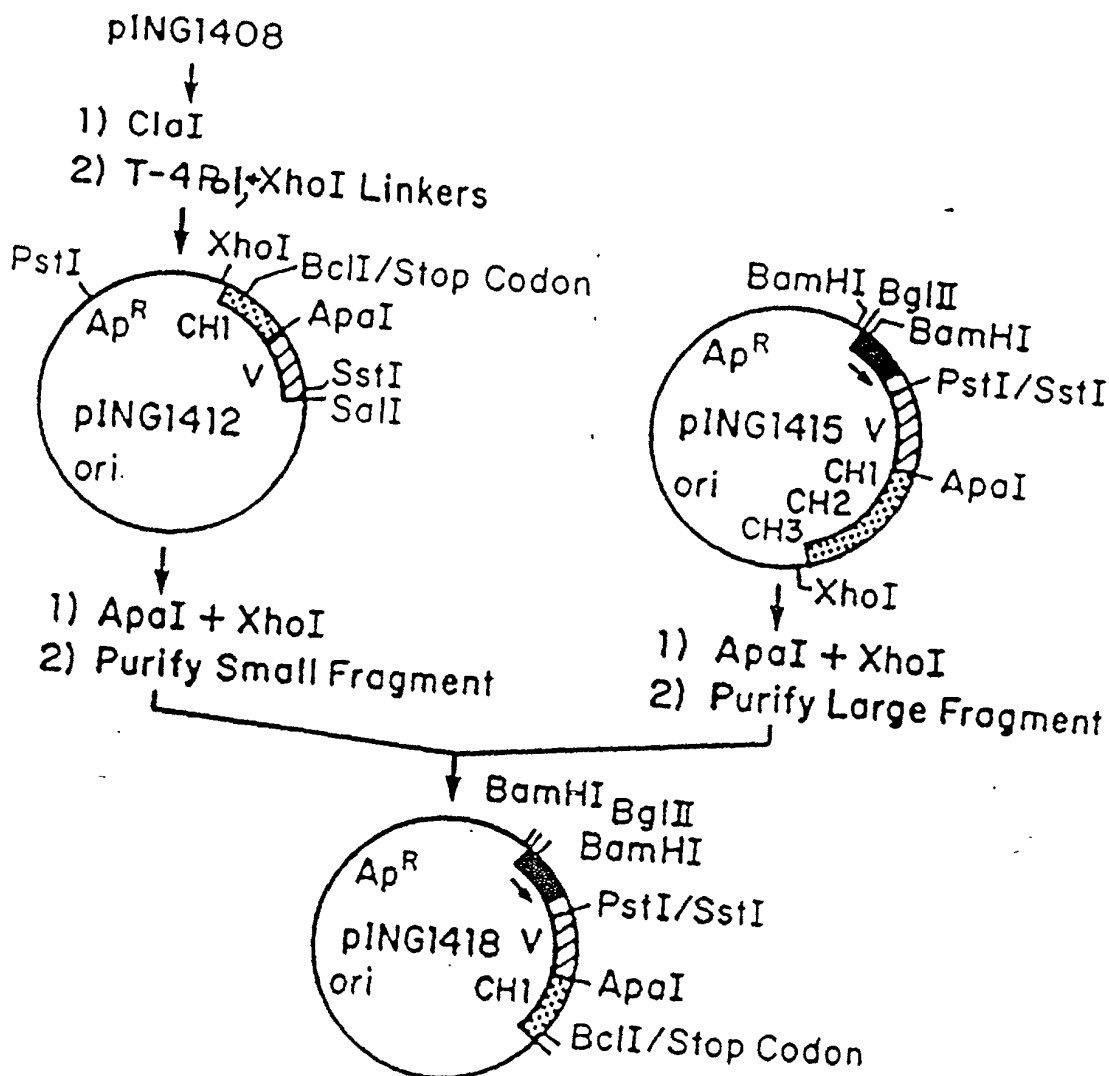


FIG. 33B

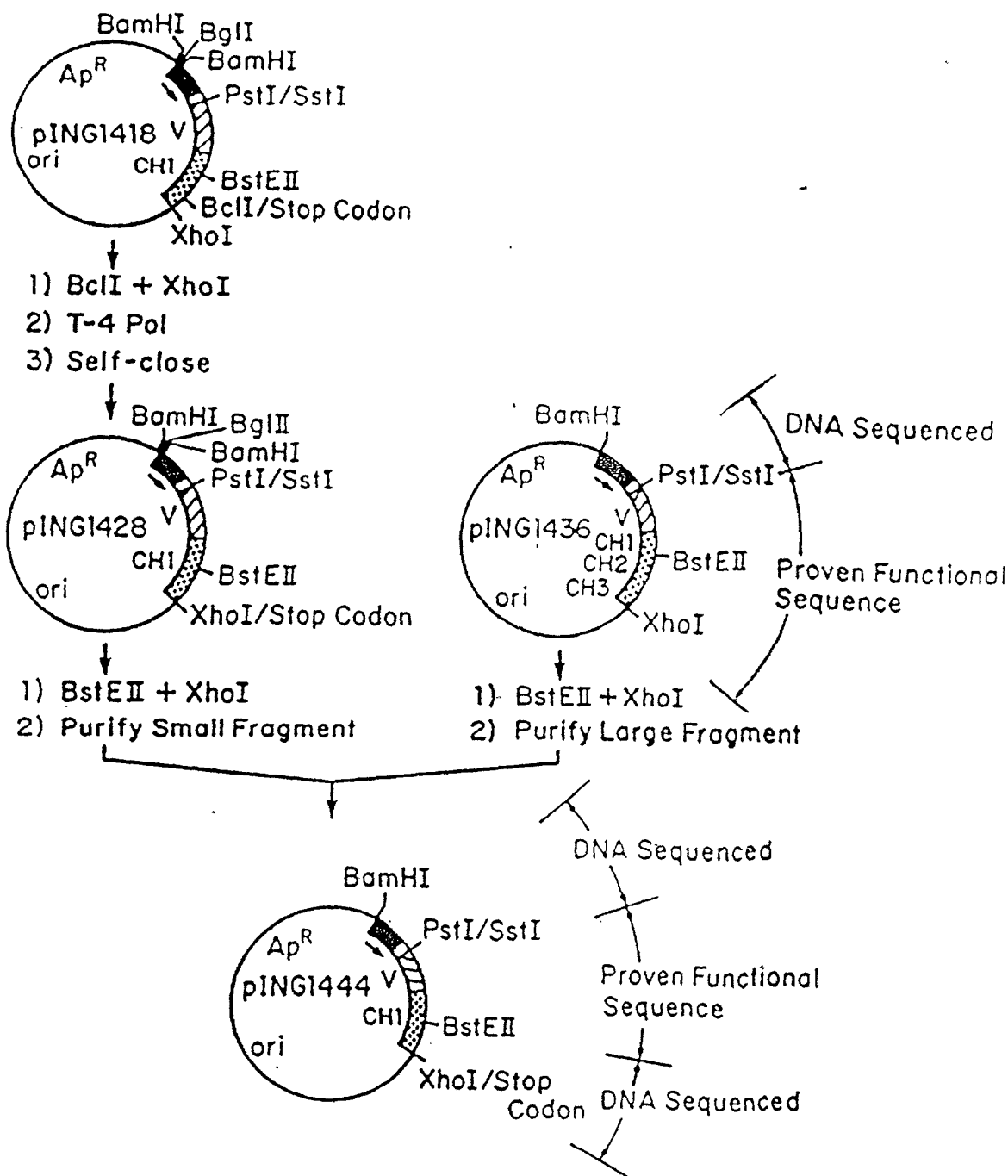


FIG. 34

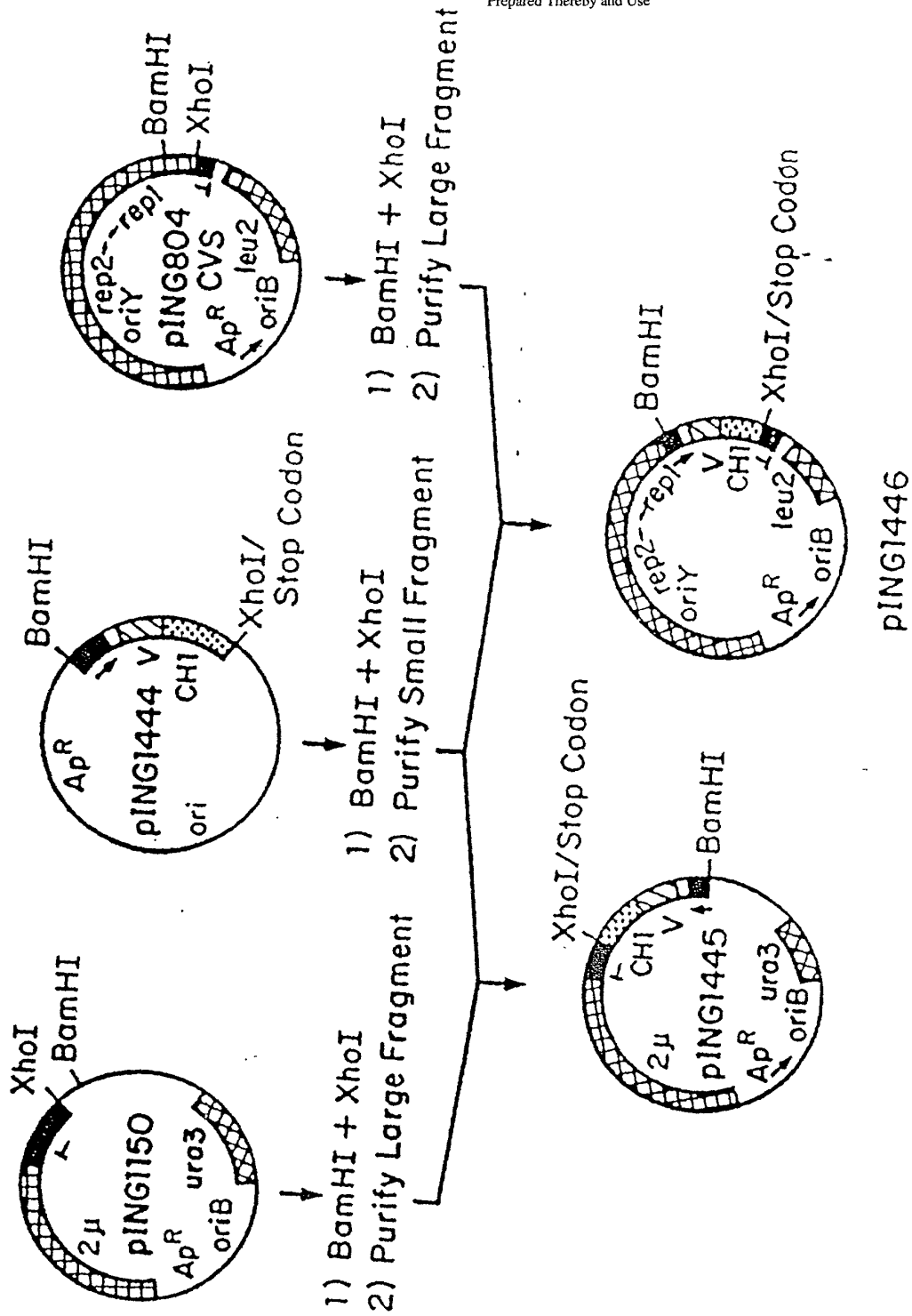


FIG. 35

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DraI

TTT AAA AGG AAA TTT TTT CTT ATA AAA

CCC AAA TTA TCC AAT CAT CAG TAT TAC AAA ATG TTT CAA CCG TAA TAC ATT TAA CAT TTC

ACC CTT GAA CTG ATC TTA TTT TTT GAC CAC AAT CCC CTT GGT TTT TCA CCA AAA CTG AGT

NdeI

TTC ATT TTT GTT GAA AAA TTT GTA CCT GCG ACA TCG GGC ATA TGG AAC GAT AAA TGC CCA

1

TGA AAA TTC TAT TTC AAG GAG ACA GTC ATA ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT
 MET Lys Tyr Leu Leu Pro Thr Ala Ala Ala

HaeIII

90

Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala MET Ala Ala Asn Thr Gly Gly Tyr Ala Thr
 GGA TTG TTA TTA CTC GCT GCC CAA CCA GCG ATG GCC GCA AAT ACG GGT GGC TAT GCC ACC



FIG. 36 A

20250719 15:45:00

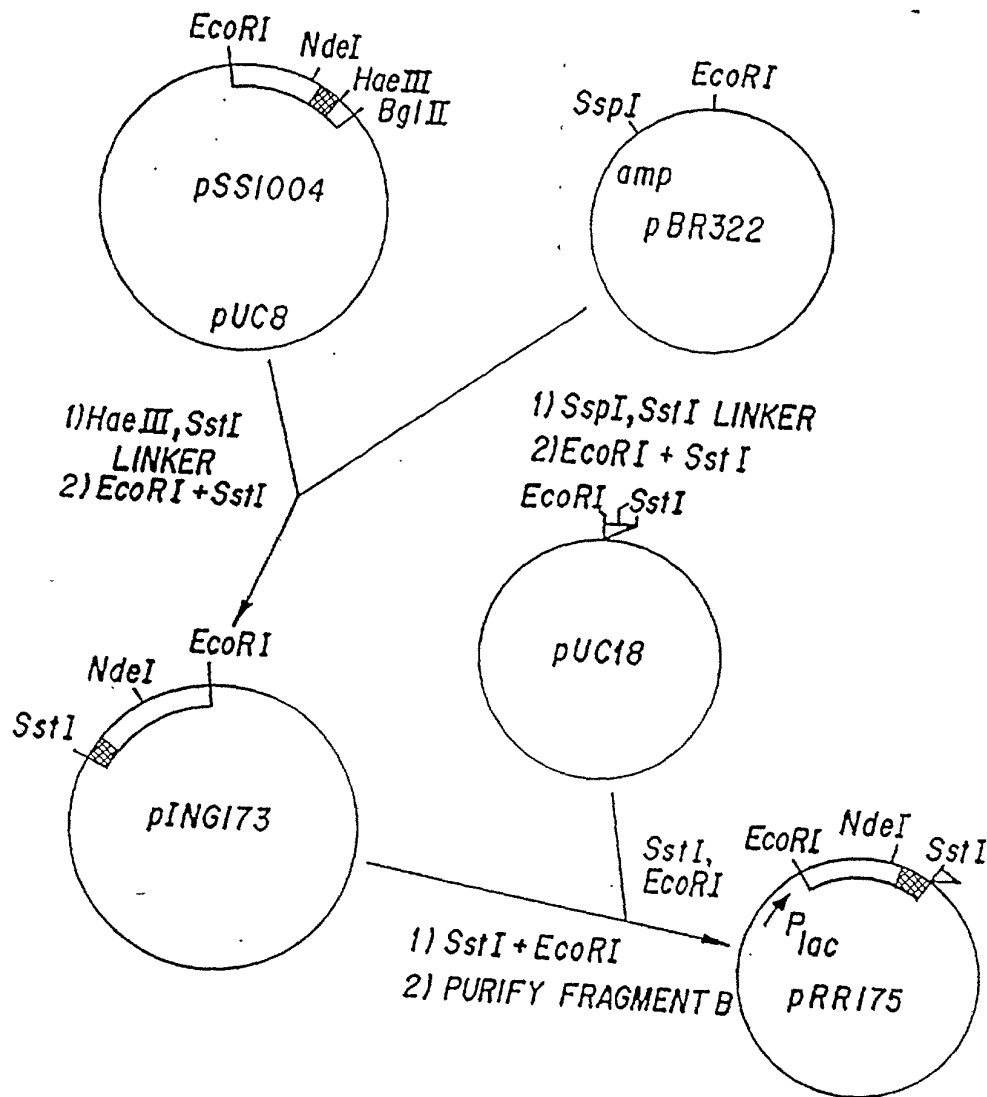


FIG. 36B

Appl. No. To Be Assigned; Group Art Unit: To Be Assigned
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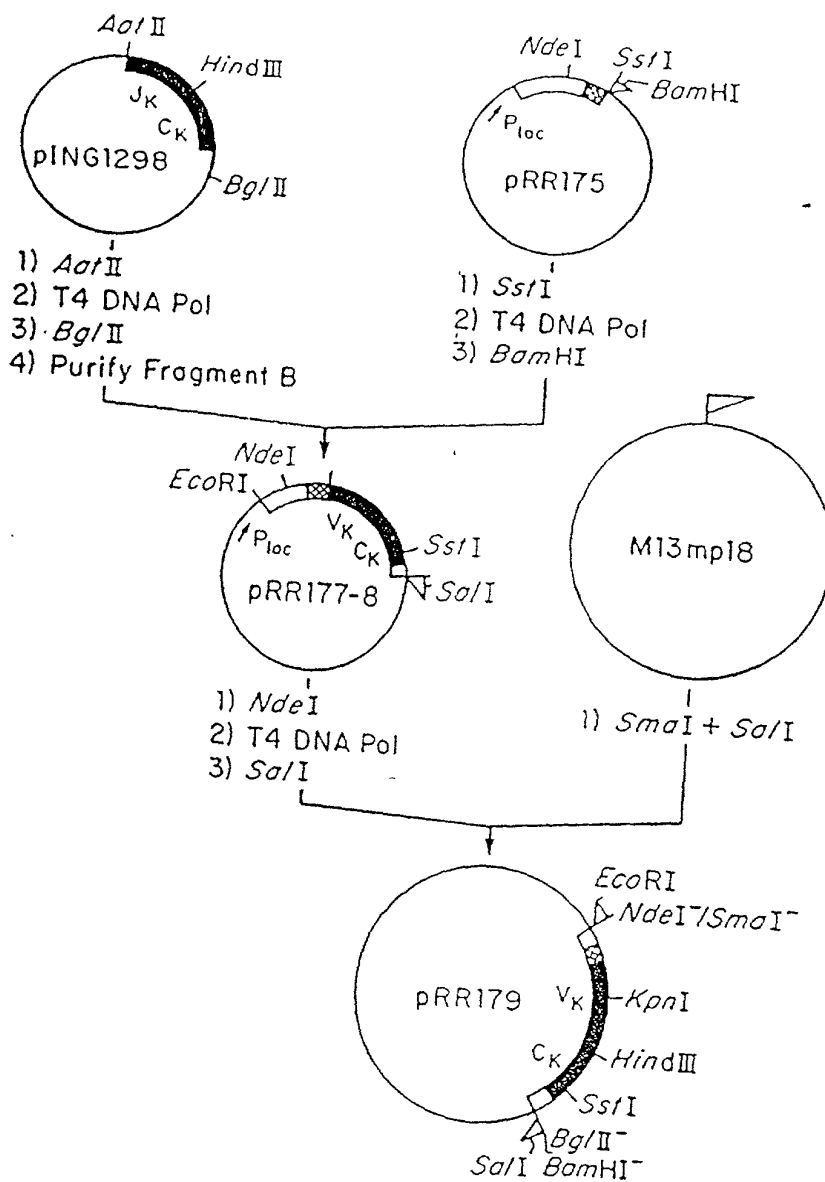


FIG. 37A

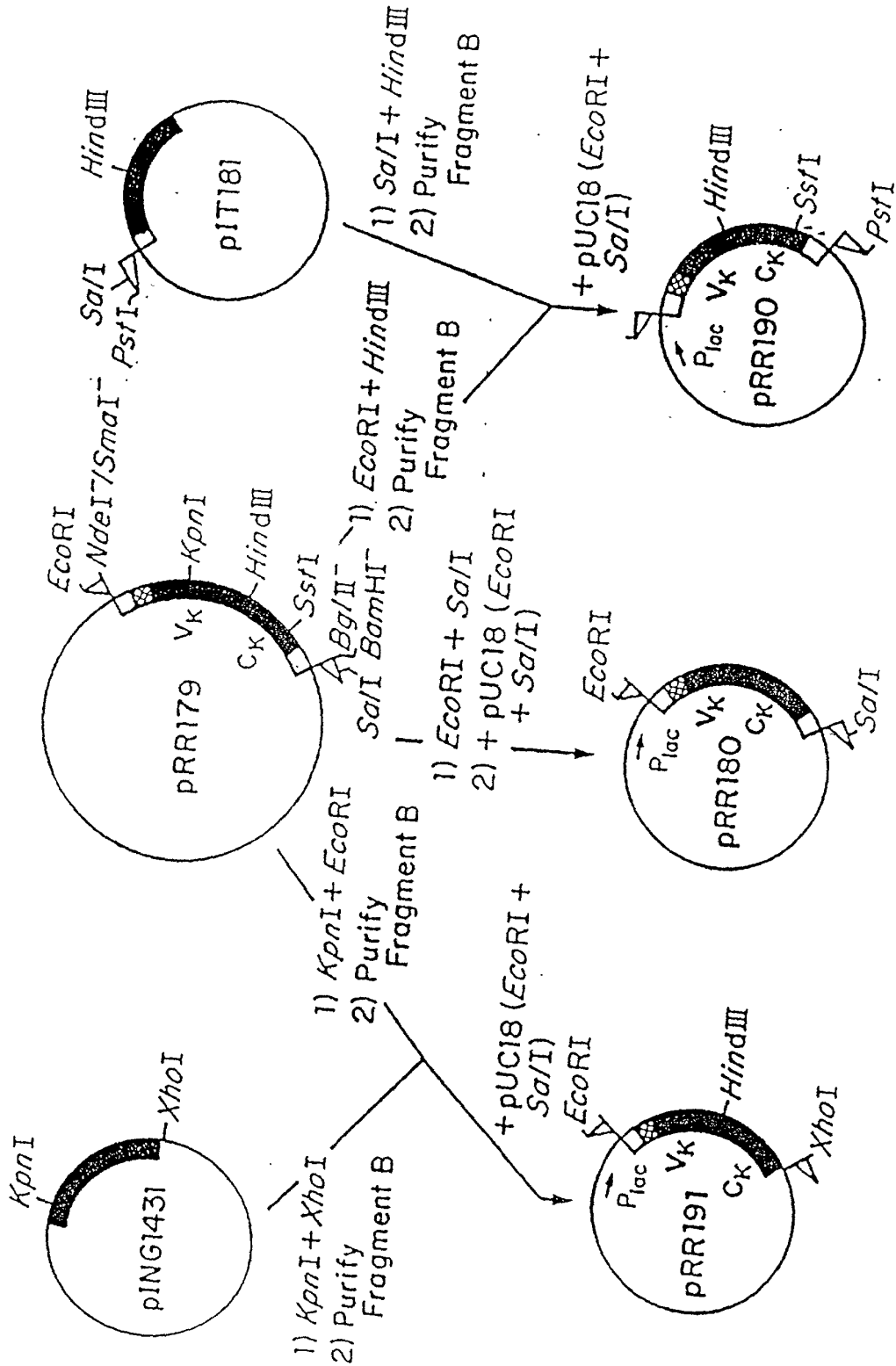


FIG. 37B

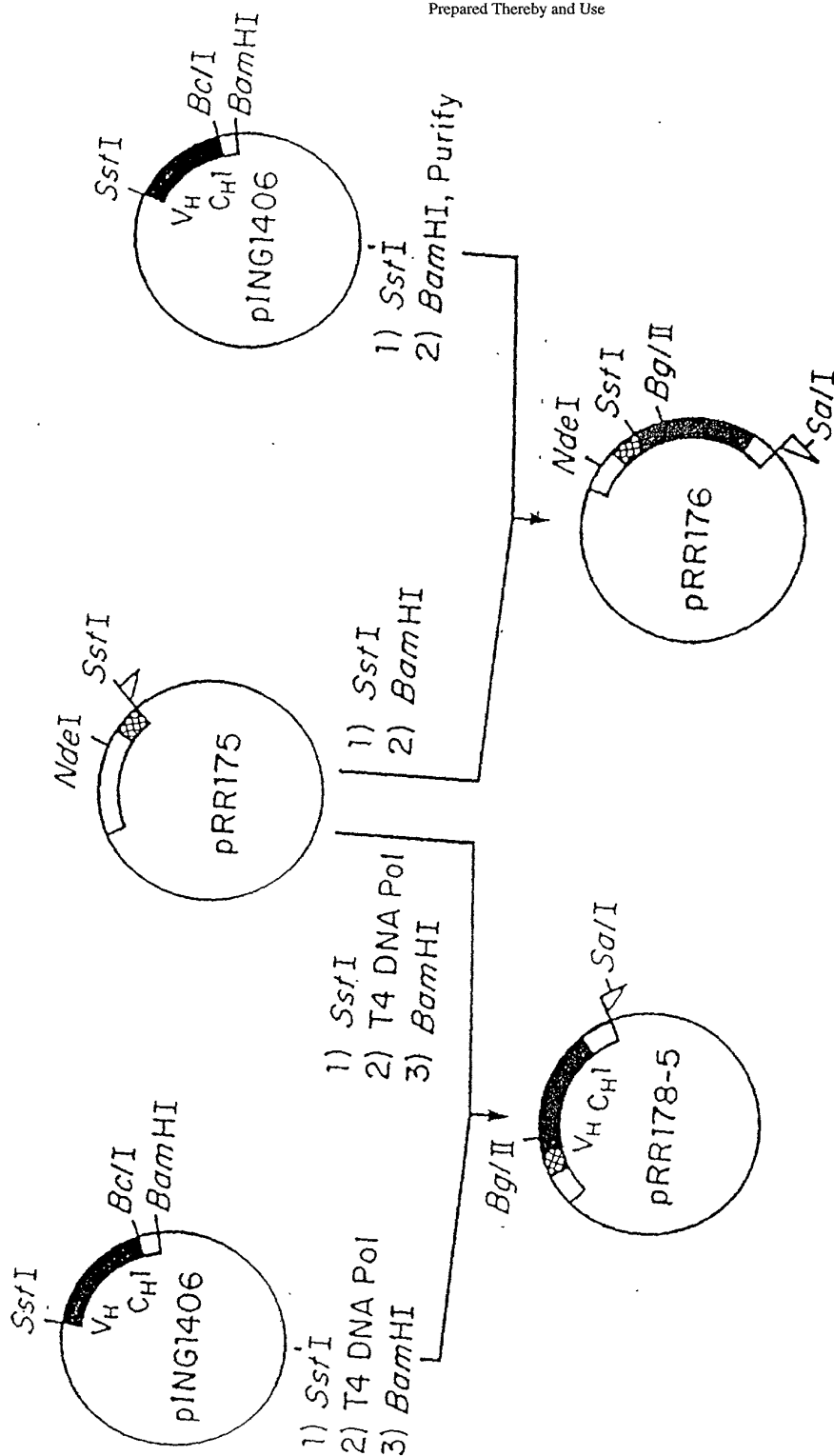


FIG. 38A

205070" 54604007

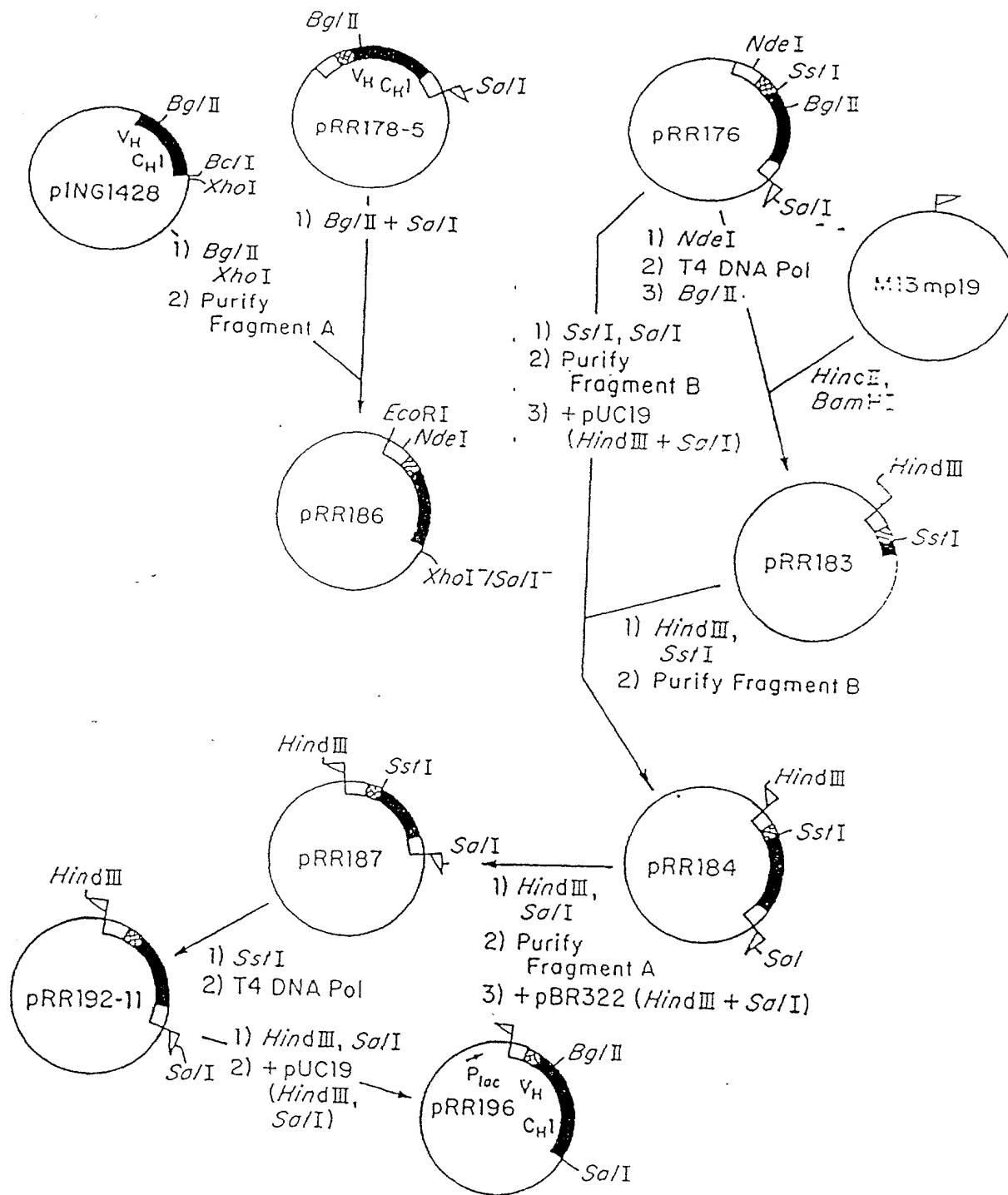


FIG. 38B

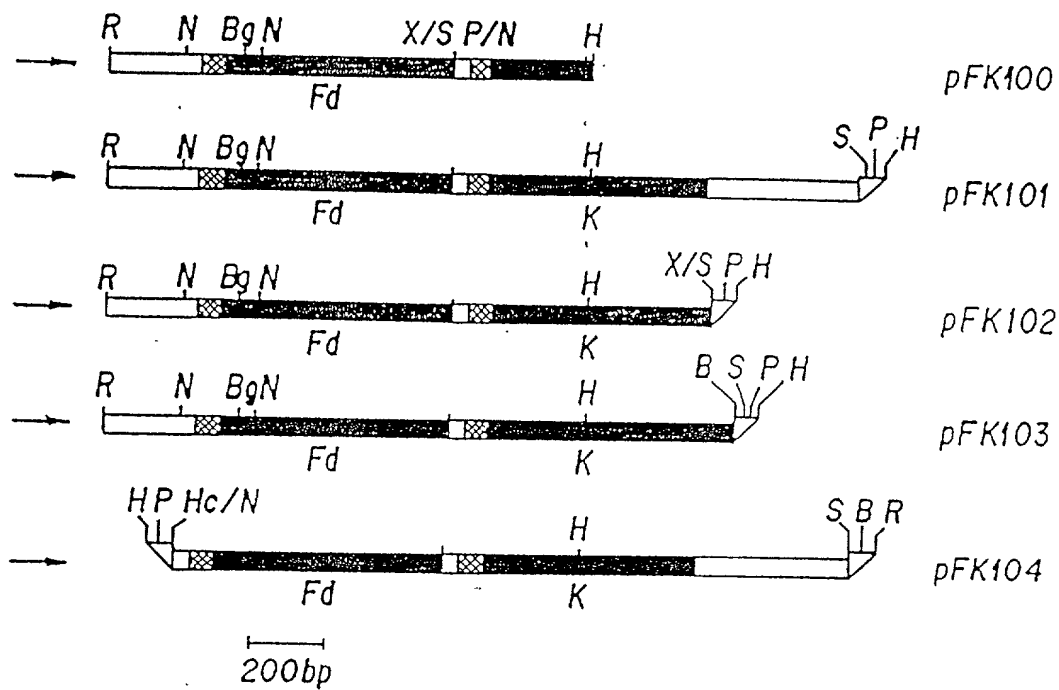


FIG. 39

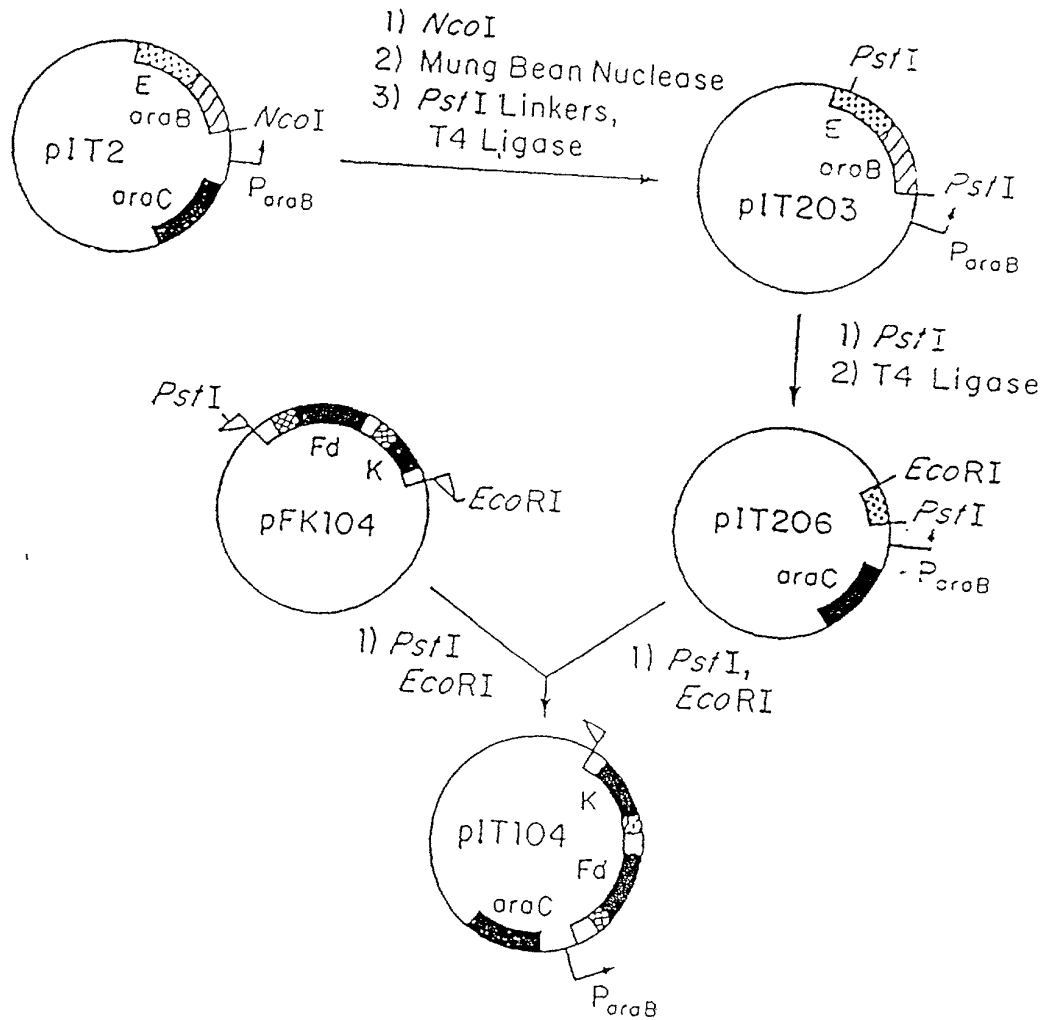
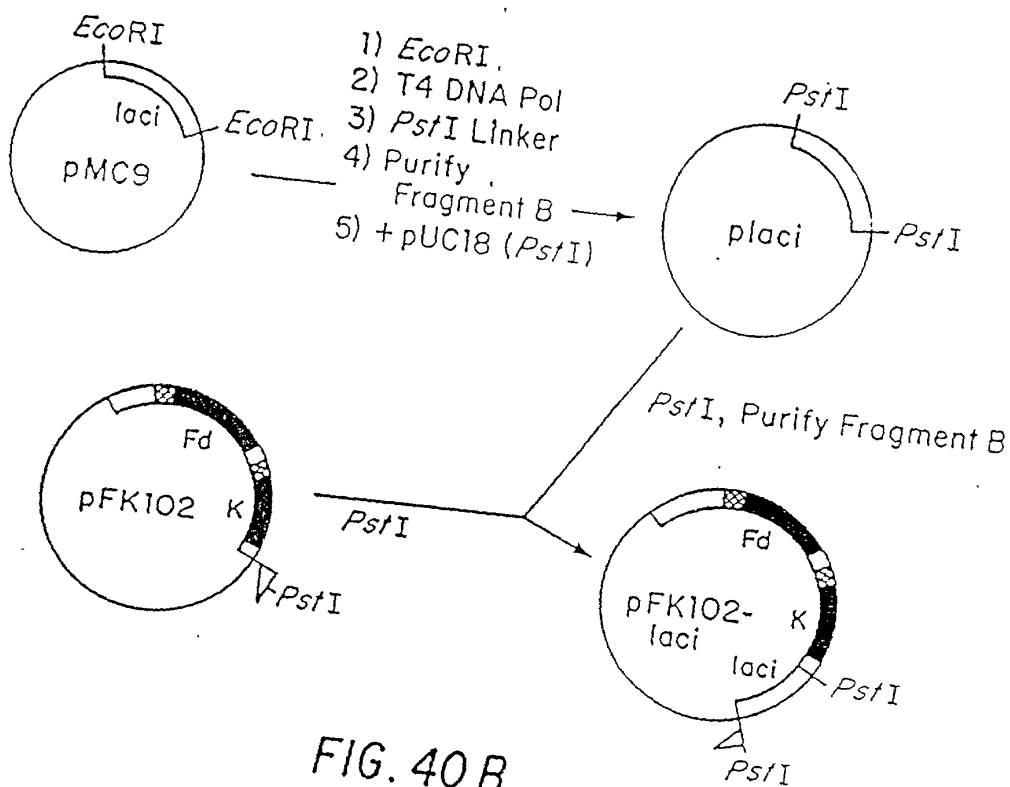


FIG. 40A

Appl. No. To Be Assigned; Group Art Unit: To Be Assigned
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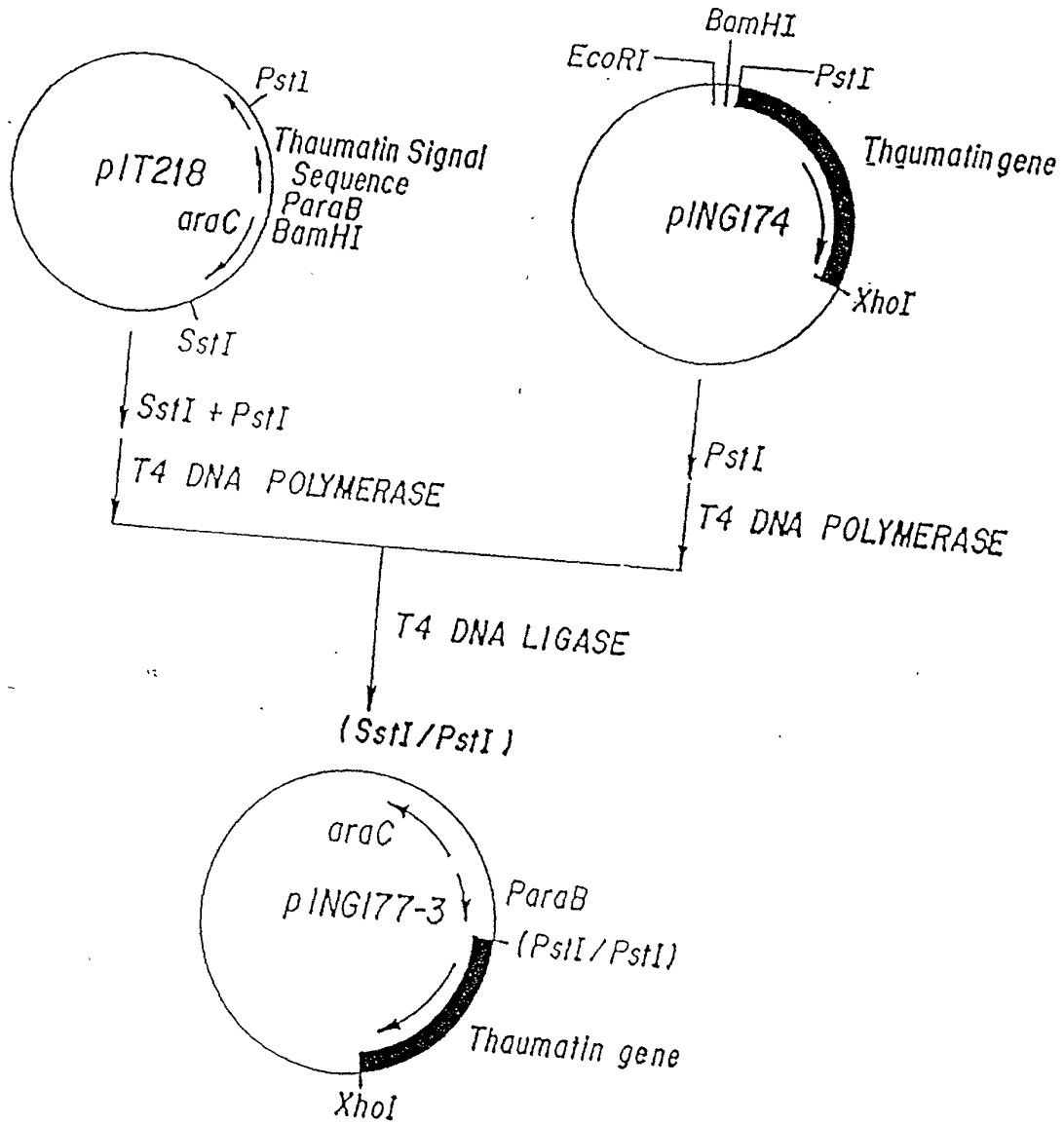


FIG. 41

206070-4604001

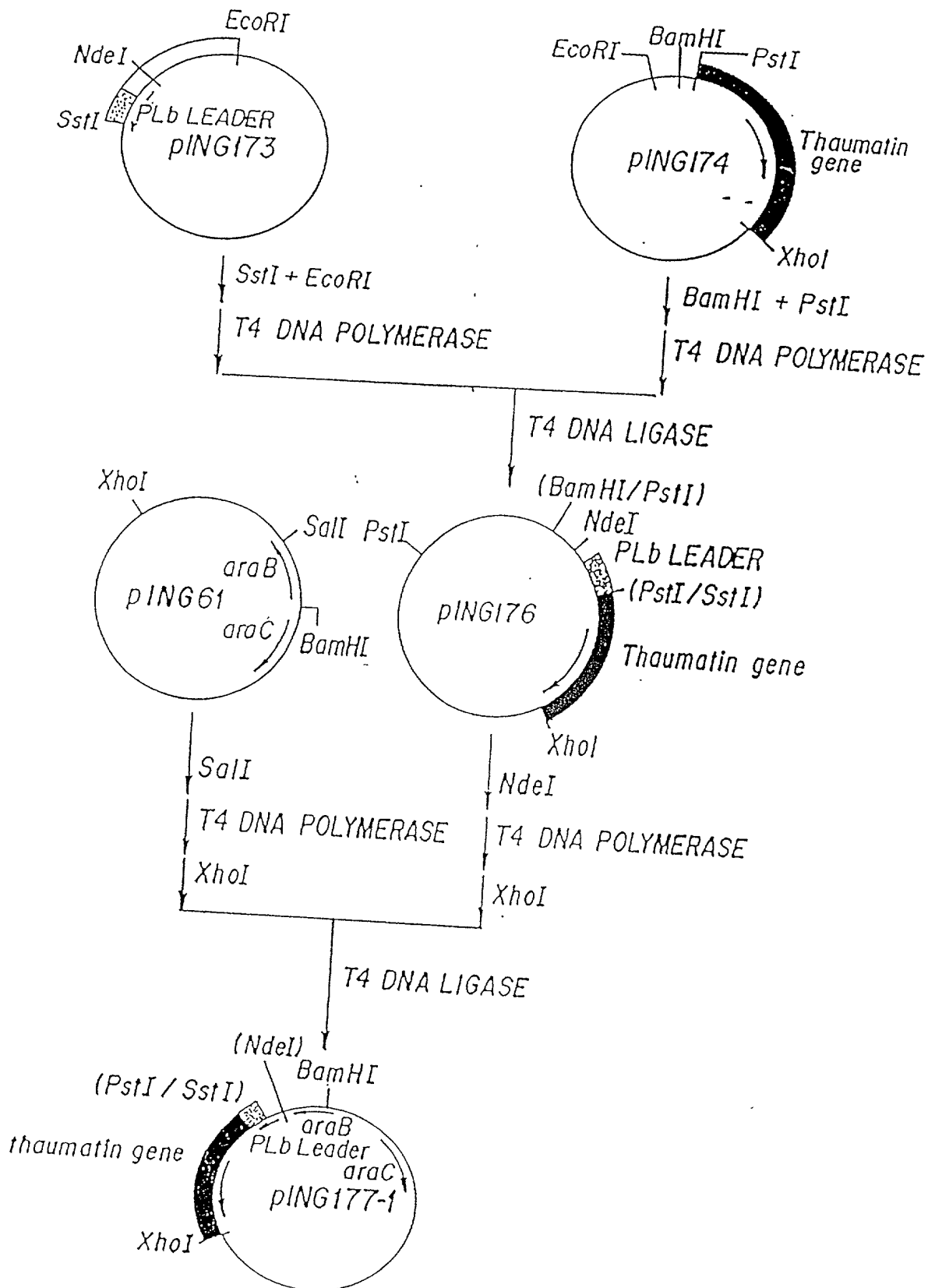


FIG. 42

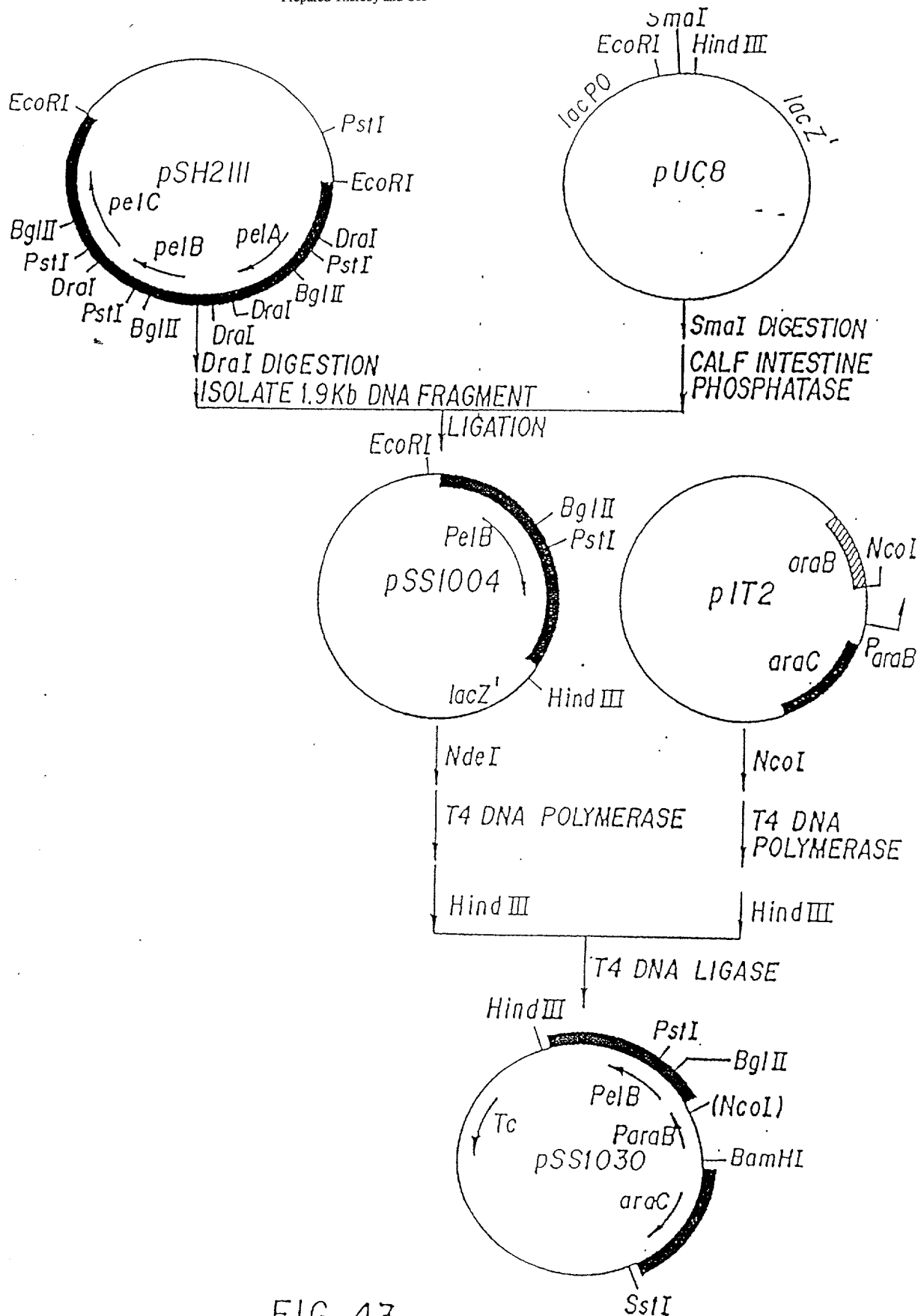


FIG. 43

206070-54604007